FACIO-SCAPULO-HUMERAL DYSTROPHY: MECHANISMS AND THERAPY APPROACHES

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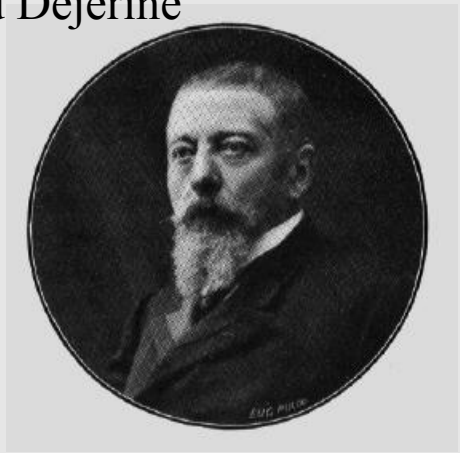
FACIO-SCAPULO-HUMERAL MUSCULAR DYSTROPHY

→ FSHD was first described in 1885 by Landouzy and Déjerine

Louis Théophile Joseph Landouzy
(1845-1917)



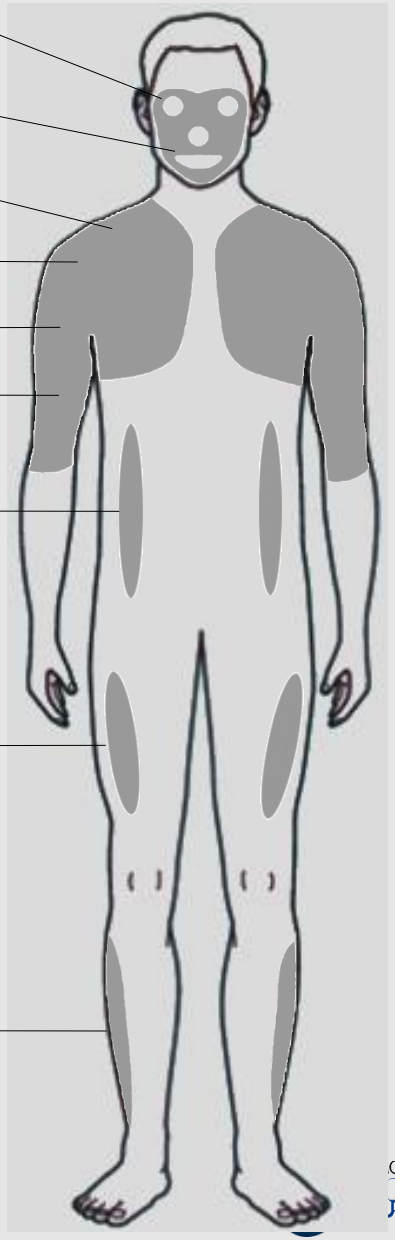
Jules Déjérine
(1849 - 1917)

→ FSHD is a hereditary neuromuscular dystrophy most frequent in France with an incidence of 1 : 14 000

→ FSHD has a variable severity and age onset, but by the age of 20, the penetrance of the disease is almost complete

→ The disease is characterized by progressive weakness and atrophy of the facial and shoulder girdle muscles, which subsequently spreads to the abdominal and pelvic girdle muscles with highly variable expressions

CLINICAL MANIFESTATIONS OF FSHD



shoulder girdle

pelvic girdle

www.med.yale.edu

www.eurasnet.info

FSHD FEATURES AT THE CELLULAR LEVEL

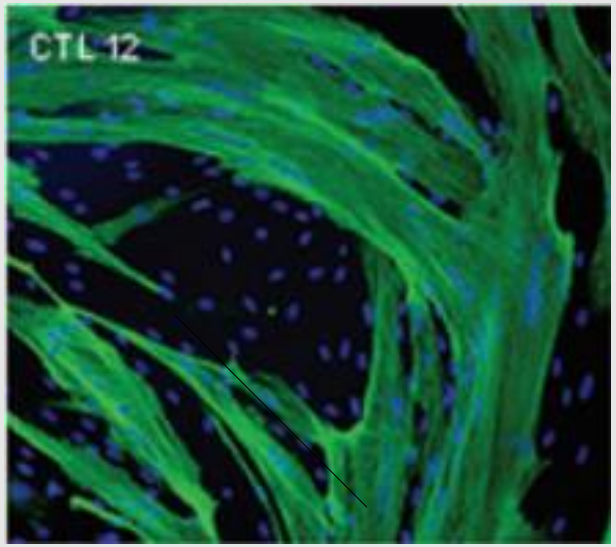
Morphology of FSHD myotubes

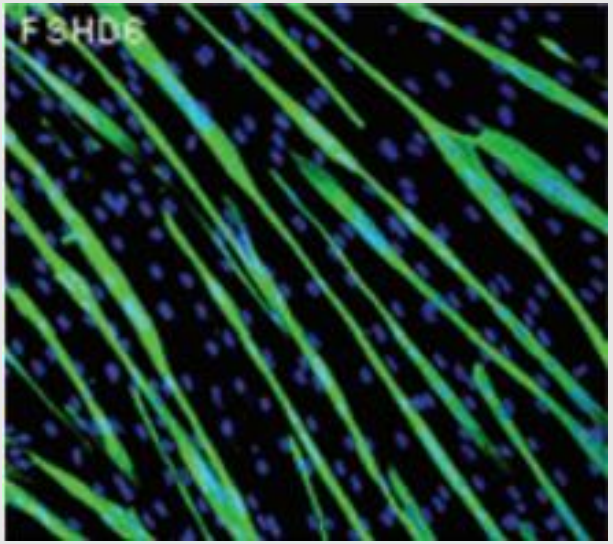
FSHD

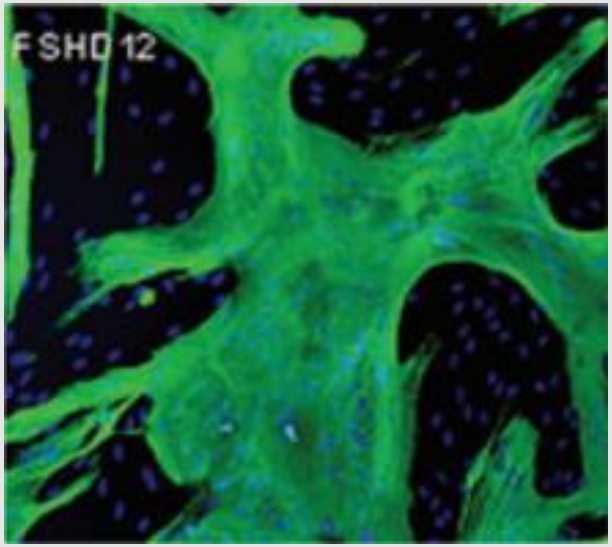
Normal

Atrophic

Disorganized

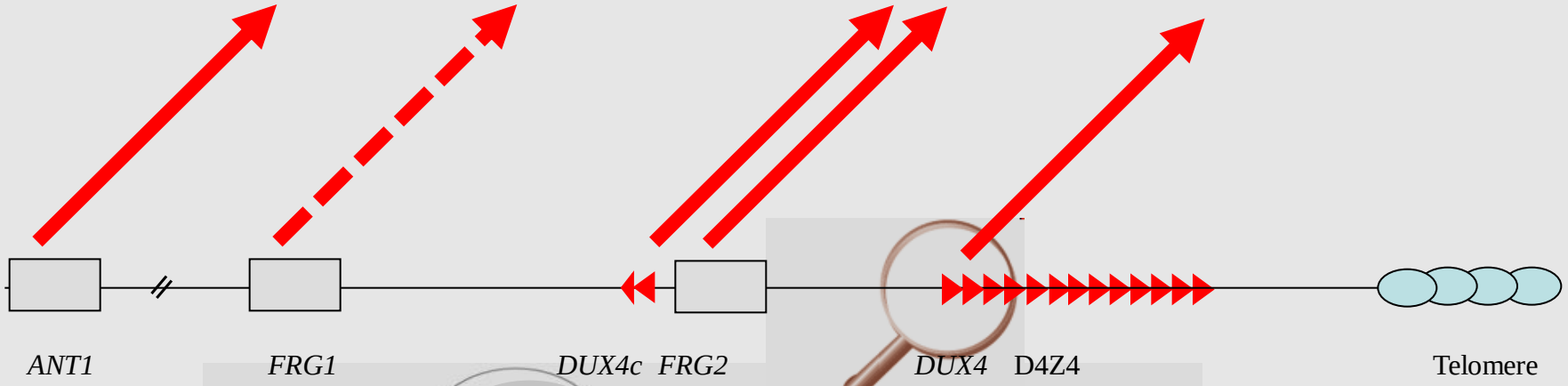


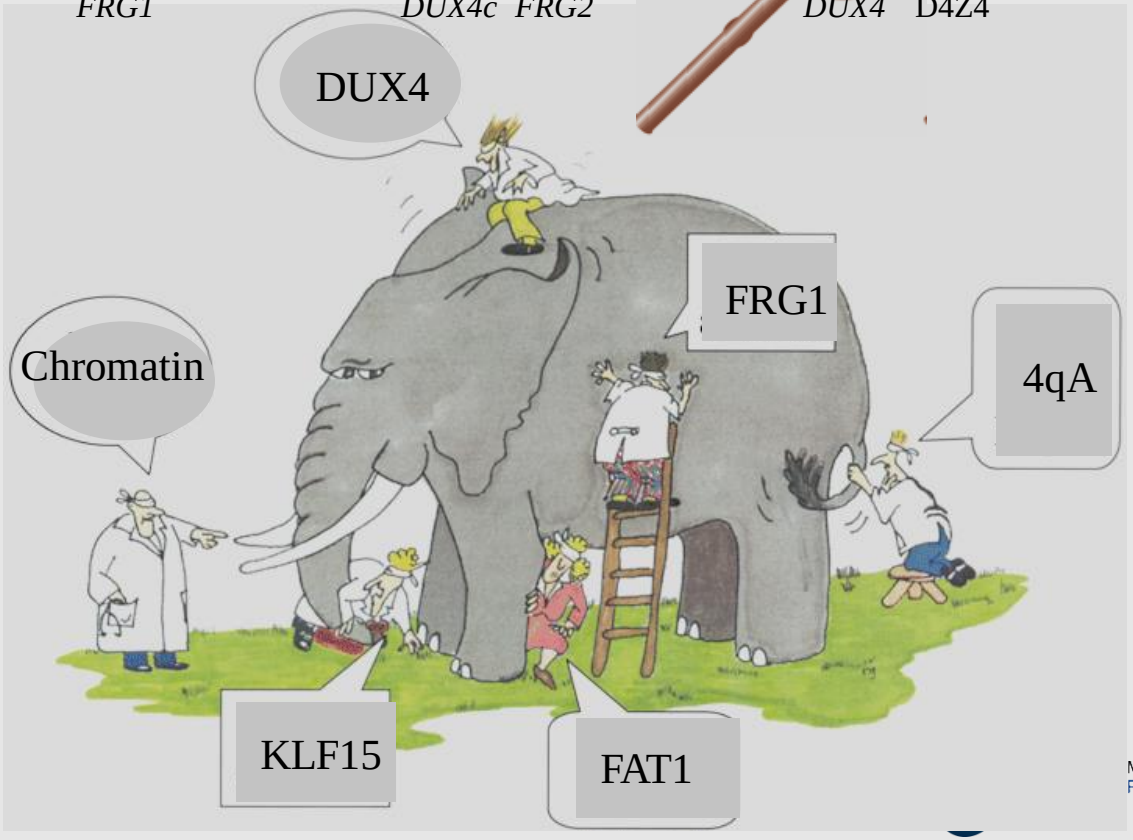




Barro et al. ***JCMM****, 2010*

D4Z4 AND THE TRANSCRIPTIONAL CONTROL IN FSHD1

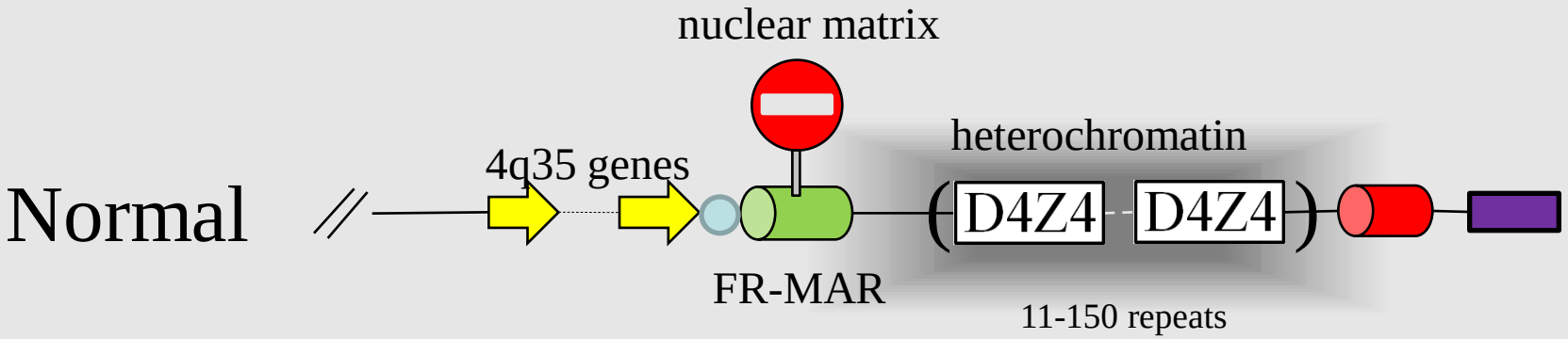




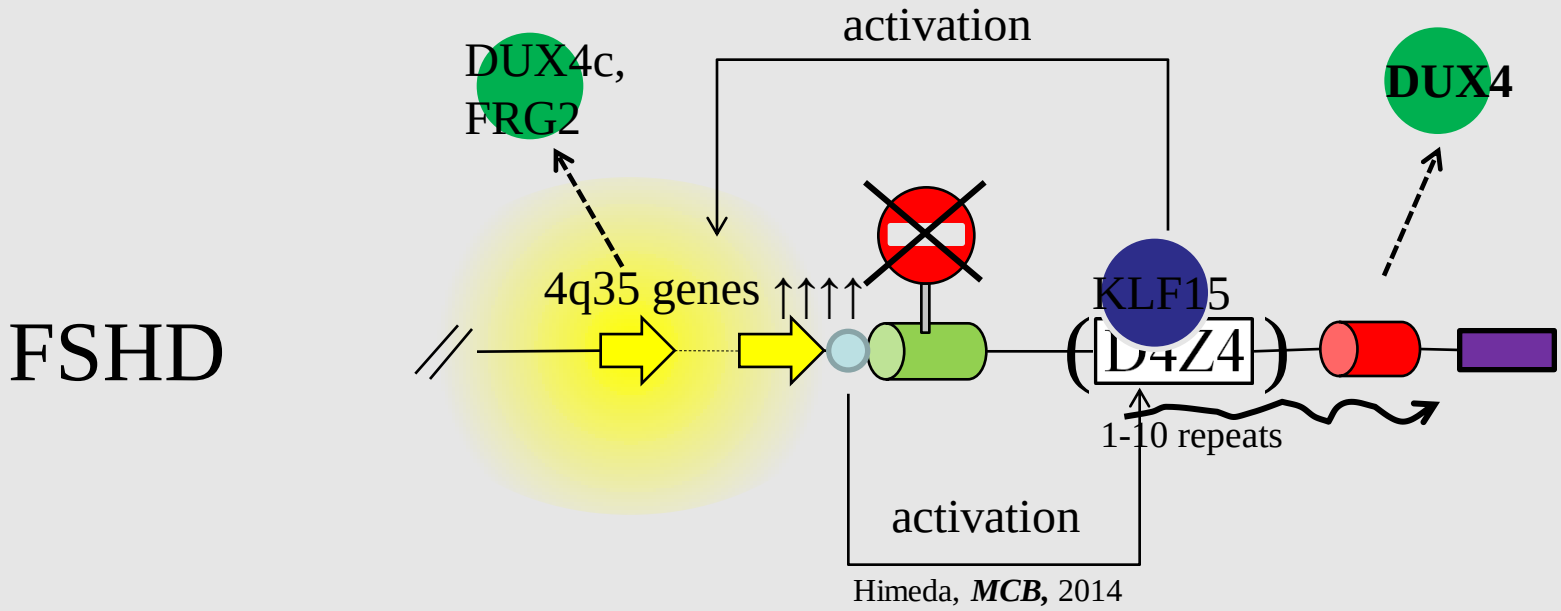
MEDECINE PARIS-SUD

Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

MOLECULAR MECHANIMS OF TRANSCRIPTIONAL CONTROL IN FSHD1



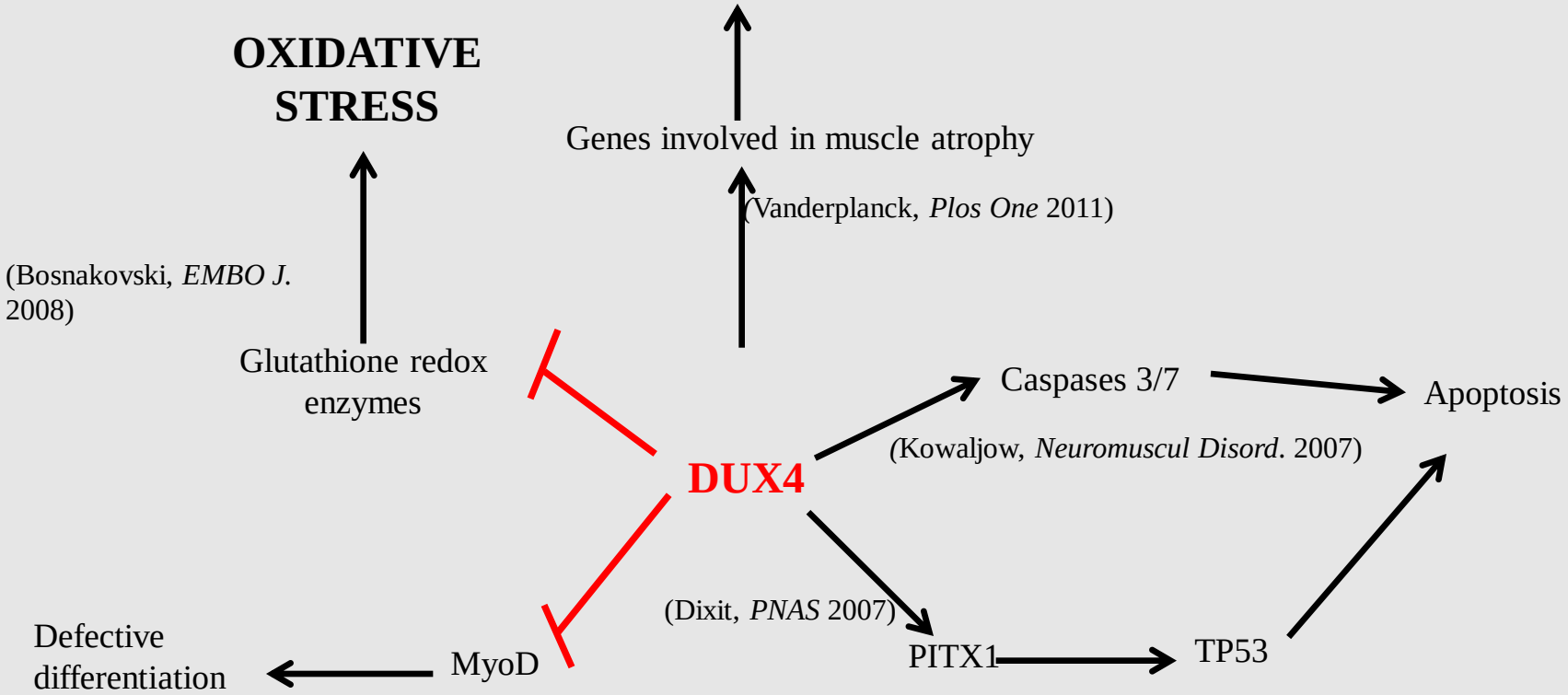
FR-MAR: FSHD-related Matrix Attachment Region



Petrov, ***PNAS***, 2006; ***Genome Res***, 2008,
Dmitriev, ***JBC***, 2013; Kiseleva, ***EJHG*, 2014**

DUX4: A CANDIDATE GENE FOR FSHD

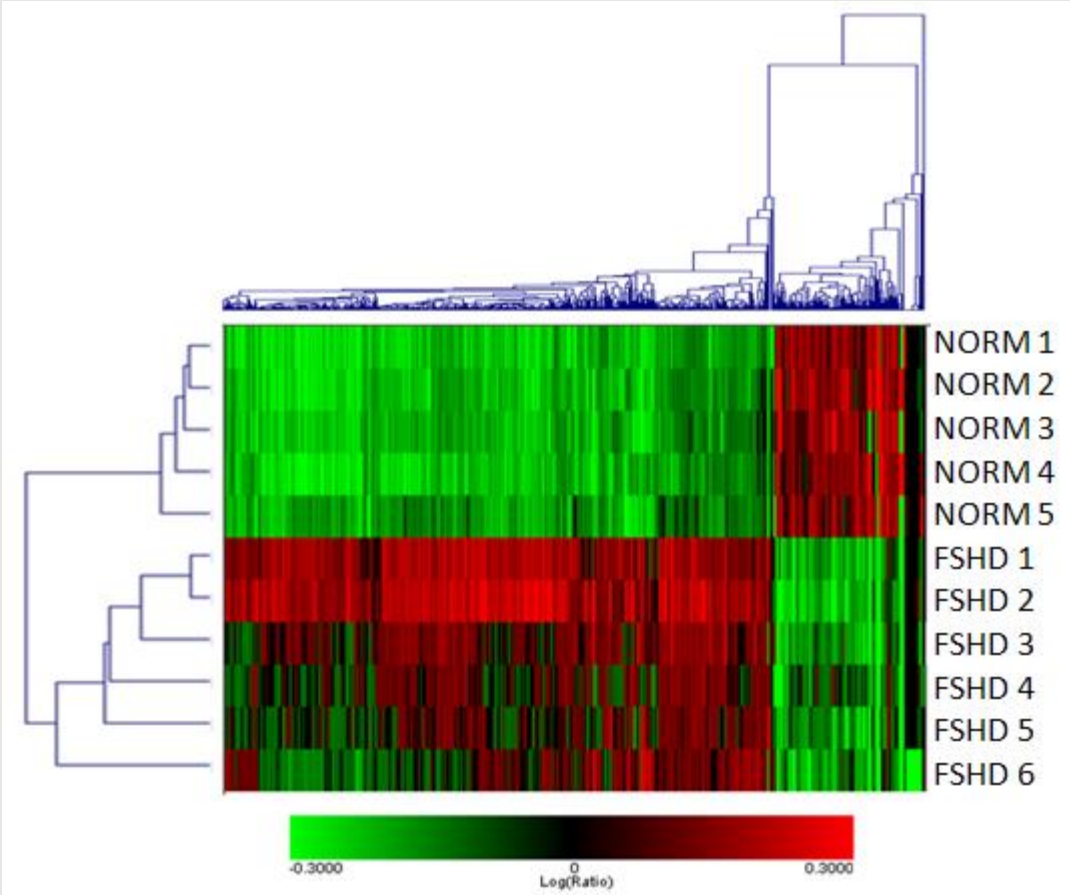
- Belongs to DUX proteins family with double homeodomains (Beckers, *Gene* 2001)
- Transcription factor containing conserved DNA binding domains at the N-terminal region
- Epigenetically repressed in somatic tissues, except in germ cells of human testes (Snider, 2010)
- **Effects :**



DNA REPAIR GENES ARE UPREGULATED IN FSHD MYOBLASTS

Upregulated in FSHD

FANCD2:	3,1x
BRCA2:	3,8x
BRCA1:	2.3x
Rad51:	3,0x
ERCC1:	
MSH6:	
Rad50:	



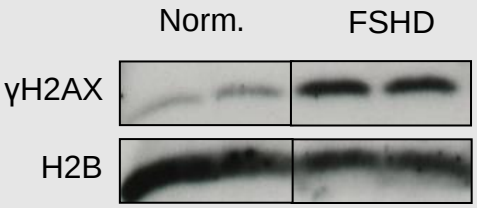
DNA DAMAGE AND OXDATIVE STRESS: A ROLE IN FSHD?

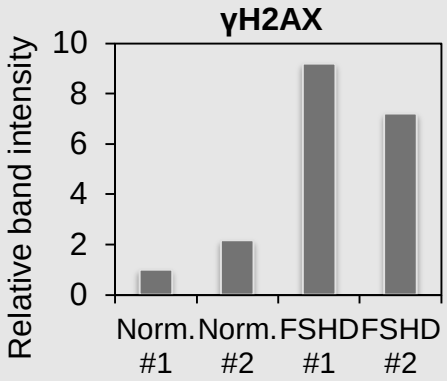
→Does oxidative stress affect DNA in FSHD myoblasts?

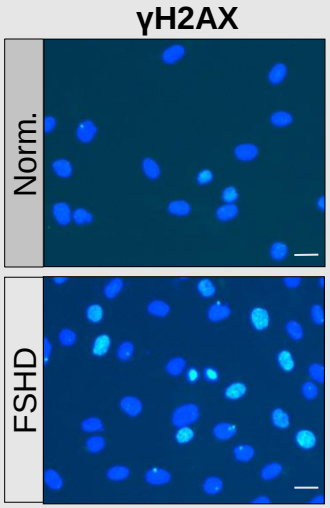
→Is there a link between DUX4, oxidative stress and DNA damage in FSHD?

→What is the effect of oxidative stress on pathophysiology of FSHD?

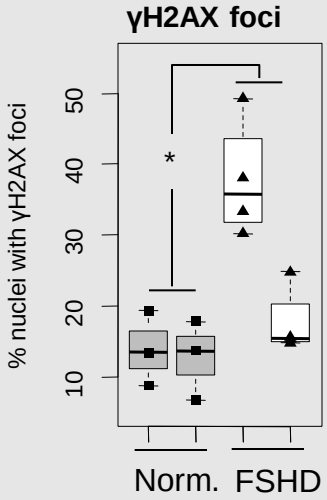
DNA DAMAGE IN FSHD MYOBLASTS





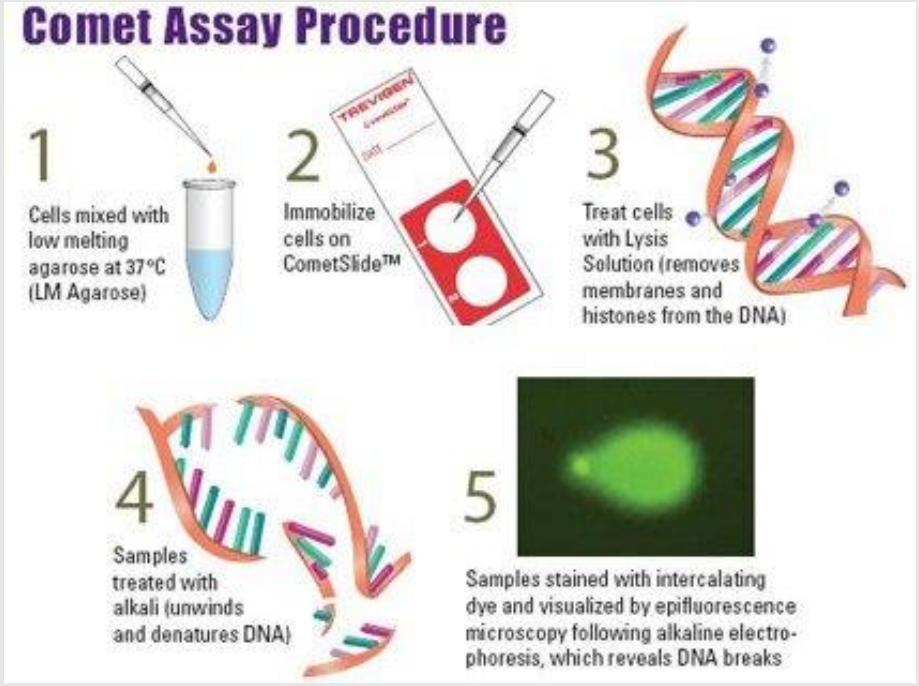


green: γH2AX (FITC)
blue: DNA (DAPI)

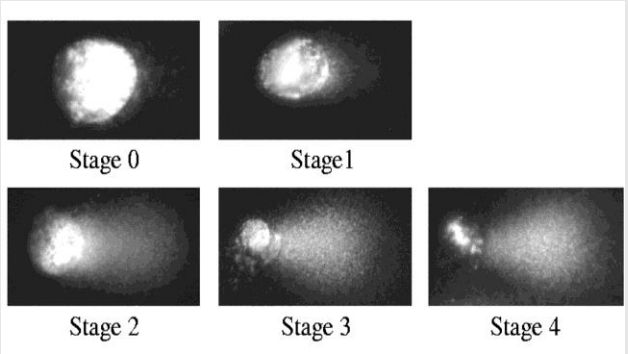


Norm. FSHD

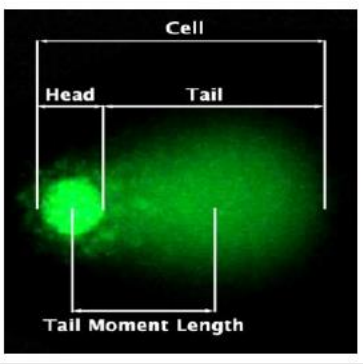
DNA DAMAGE ASSESSMENT BY COMET ASSAY TECHNIQUE



Visual analysis



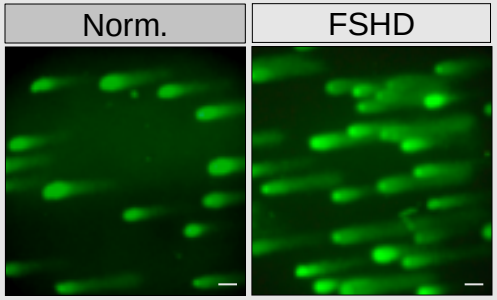
TriTek CometScore



% DNA in tail → DNA break frequency
Tail length → size of the DNA fragments

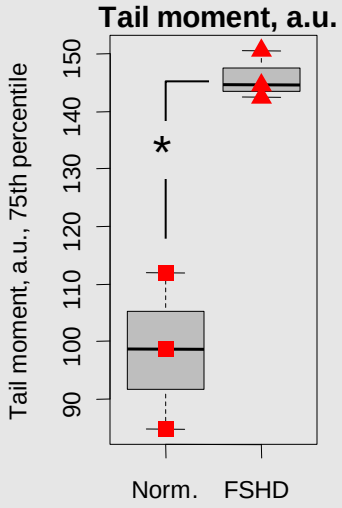
DNA DAMAGE IN FSHD PRIMARY MYOBLASTS

A



green :DNA (SybrGreen)

B



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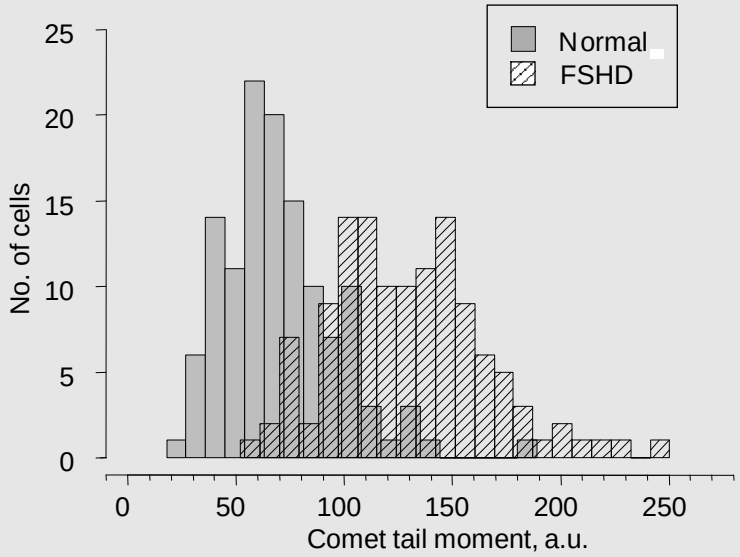
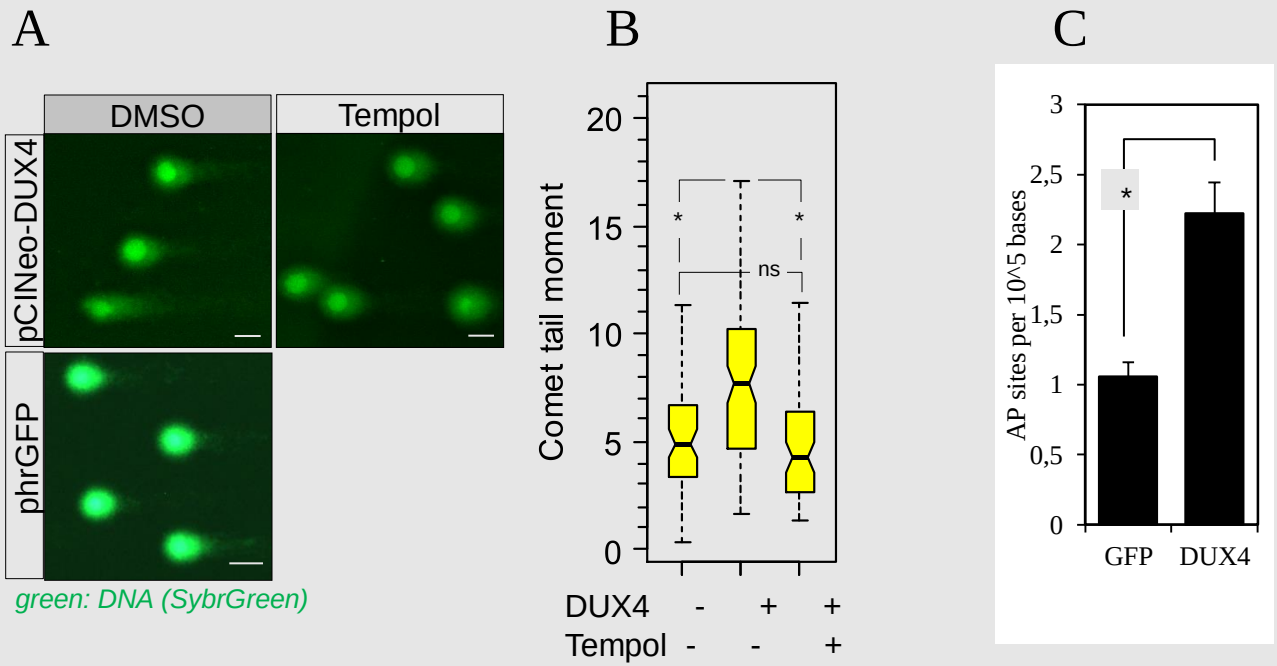


→DNA damage is higher in FSHD as compared to normal primary myoblasts

DUX4 INDUCES DNA DAMAGE IN FSHD

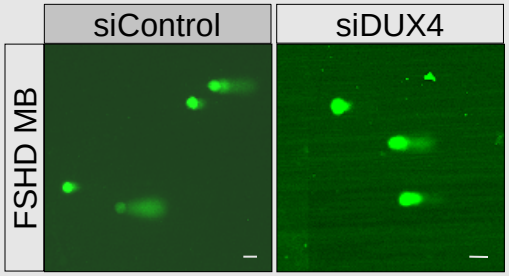
➢ **DUX4 overexpression in human immortalized myoblasts**



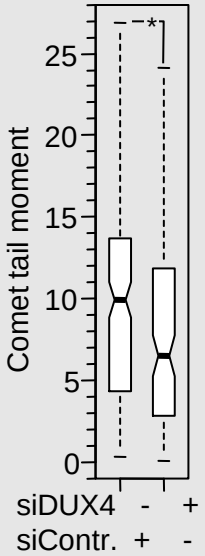
→ DNA damage was significantly higher in DUX4-overexpressing cells

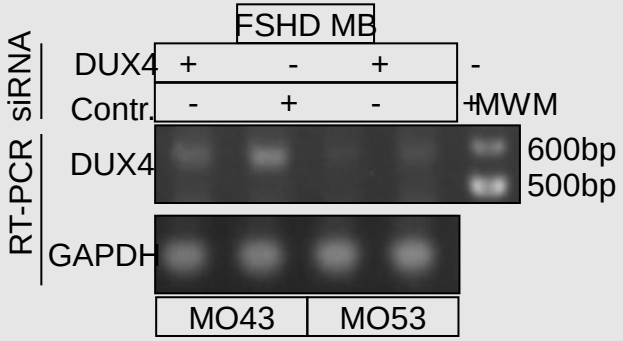
DUX4 KNOCKDOWN IN FSHD PRIMARY MYOBLASTS

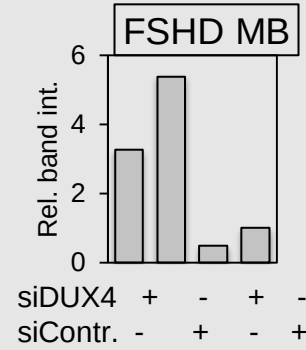
Primary FSHD myoblasts transfected with siDUX4:



Green: DNA (SybrGreen)



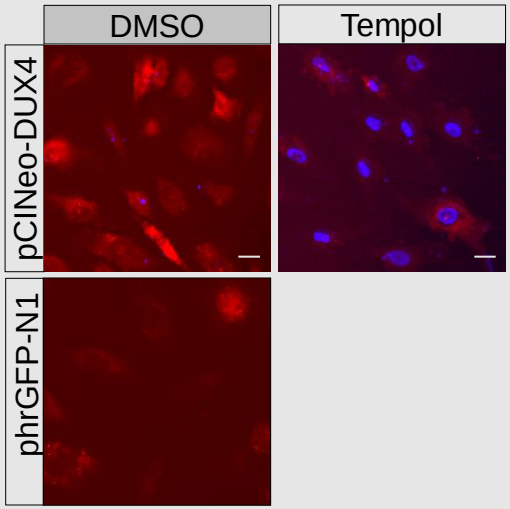




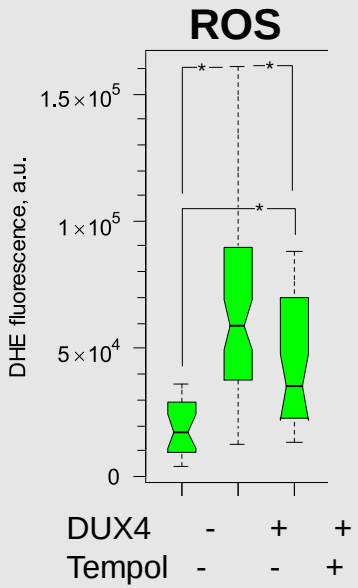
→ DUX4 knockdown reduces DNA damage in FSHD myoblasts

DUX4 OVEREXPRESSION INDUCES ROS AND CAN BE PARTIALLY COUNTERED BY TEMPOL

Measurement of the ROS level by Dihydroethidium (DHE) labeling of living cells



blue: DNA (DAPI), red: ROS (DHE)

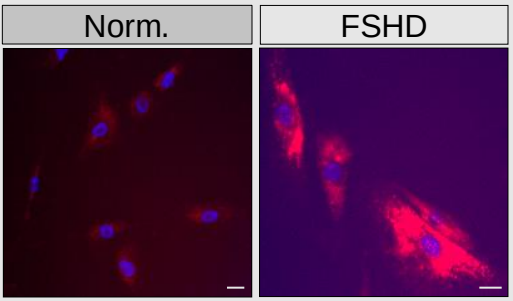


→ROS level was significantly increased in DUX4-overexpressing immortalized myoblasts

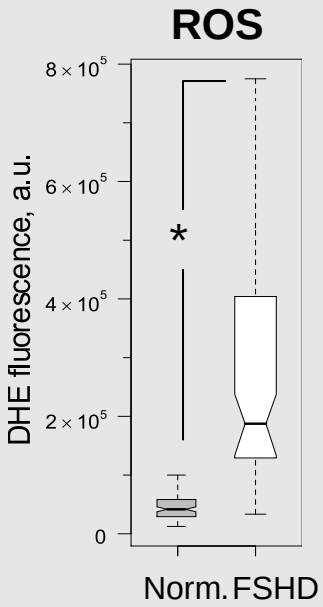
→A synthetic anti-oxidant molecule (Tempol) reduced DUX4-induced DNA damage and ROS accumulation in DUX4-transfected cells

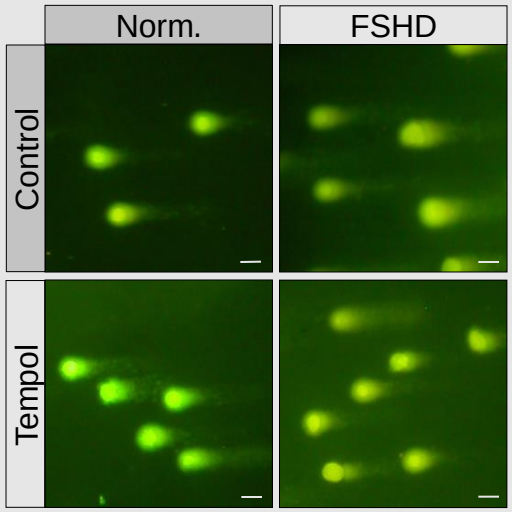
→ DNA damage by DUX4 involves ROS accumulation in the cell

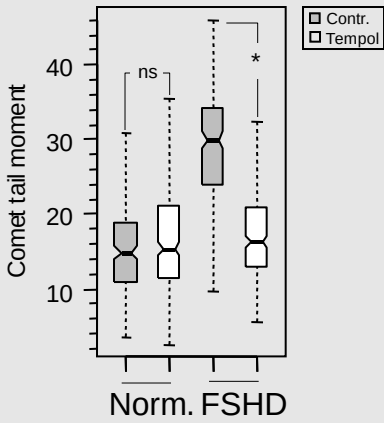
DNA DAMAGE IN FSHD MYOBLASTS IS PROVOKED BY ROS



blue: DNA (DAPI), red: ROS (DHE)



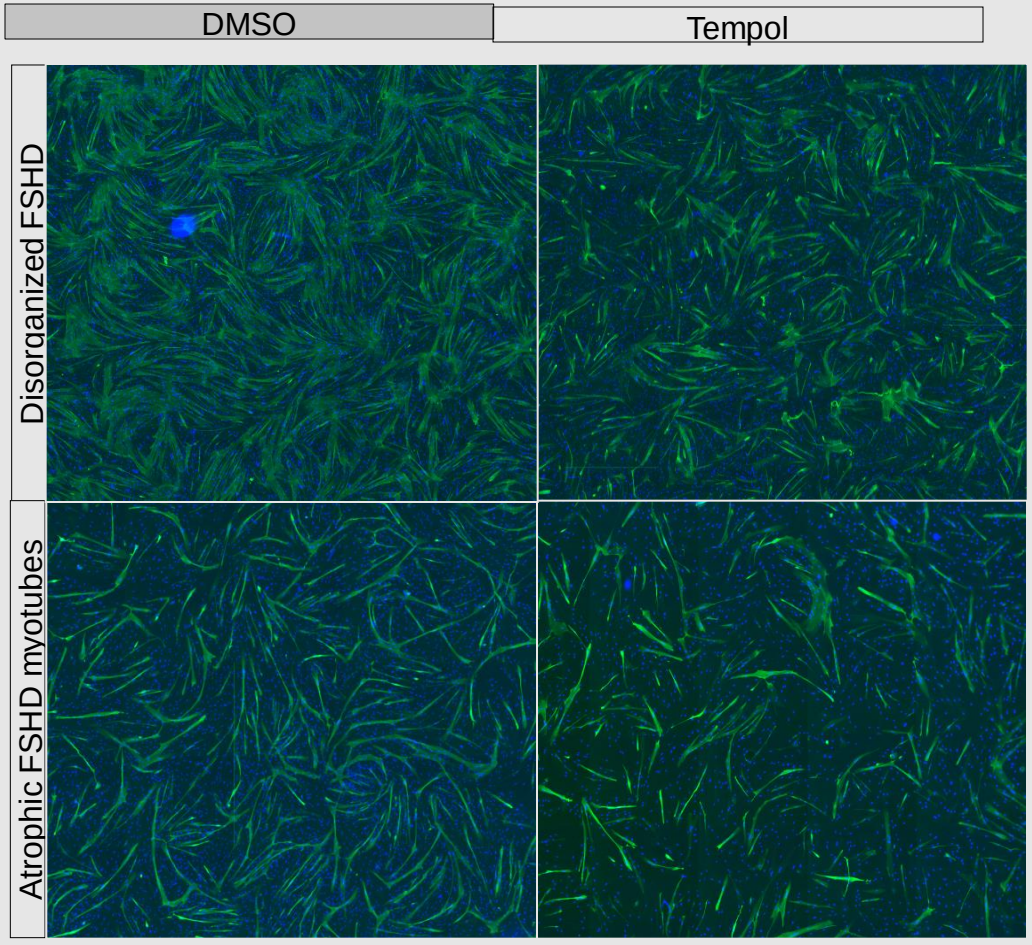


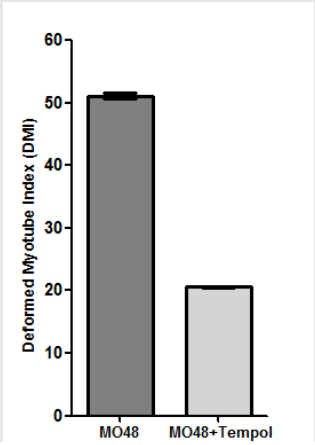


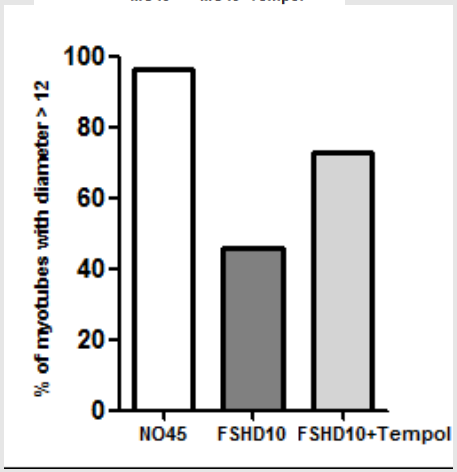
→The level of ROS was considerably higher in FSHD myoblasts

→Tempol-treated FSHD cells demonstrated a level of DNA damage similar to that of normal cells

DNA DAMAGE AFFECTS THE MYOGENIC DIFFERENTIATION OF FSHD MYOBLASTS







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- **Evgeny Sheval,** MSU, Moscow
- **S. Bury-Moné,** ENS Cachan

- Evaluation of the effect of Tempol on myogenic differentiation of atrophic FSHD cell lines

- Evaluation of the effect of Tempol on myogenic differentiation of DUX-transfected myoblasts: does it correct the atrophic phenotype induced by DUX4?

- Stress ox on muscle homeostasis: article musaro 2010

- stress ox and gene regulation: MyoD, c-Abl etc

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II- Nuclear DNA in FSHD myoblasts is resistant to low doses of oxidative stress

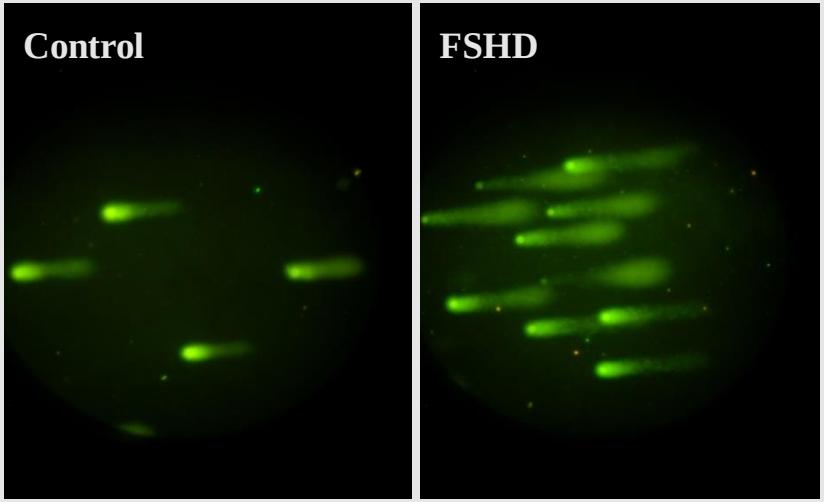
DNA repaira and replication genes are differentially expressed in FSHD myoblasts

DNA replication

Gene Symbol	Fold change	TF	TF	Replication	Mitosis	Checkp. and DDR	Oxidation	Description
BLM	2,79	E2F	MYC	X		X		Bloom syndrome, RecQ helicase-like
MCM2	1,94	E2F		X				minichromosome maintenance complex component 2
MCM3	2,66	E2F	MYC	X				minichromosome maintenance complex component 3
MCM5	2,38	E2F		X				minichromosome maintenance complex component 5
POLA	2,33	E2F		X				DNA polymerase alpha
CDK2	2,39	E2F		X				cyclin-dependent kinase 2
FEN1	1,55	E2F		X		X		flap structure-specific endonuclease 1
RRM1	1,19	E2F		X				ribonucleotide reductase M1
TK1	2,32	E2F		X				thymidine kinase 1, soluble
ERCC1	2,51	E2F				X		Excision repair cross-complementing 1
CDC2	2,42	E2F			X			cell division cycle 2, G1 to S and G2 to M
CCNA2	2,05	E2F	MYC		X			cyclin A2
TOP1	2,22		MYC	X				Topoisomerase 1
CCNB1	2,47		MYC		X			cyclin B1
RAD50	2,13		MYC			X		RAD50 homolog (S. cerevisiae)
RAD51	2,65		MYC			X		RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)
MSH2	2,21		MYC			X		mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)
MSH6	2,62		MYC			X		mutS homolog 6, colon cancer, nonpolyposis type 1 (E. coli)
EXO1	1,72		MYC			X		exonuclease 1
FANCD2	1,89		MYC			X		Fanconi anemia, complementation group D2
JUN	2,51							jun proto-oncogene
CDKN1A	2,31							cyclin-dependent kinase inhibitor 1A (p21, Cip1)

DMITRIEV Petr

Figure 1



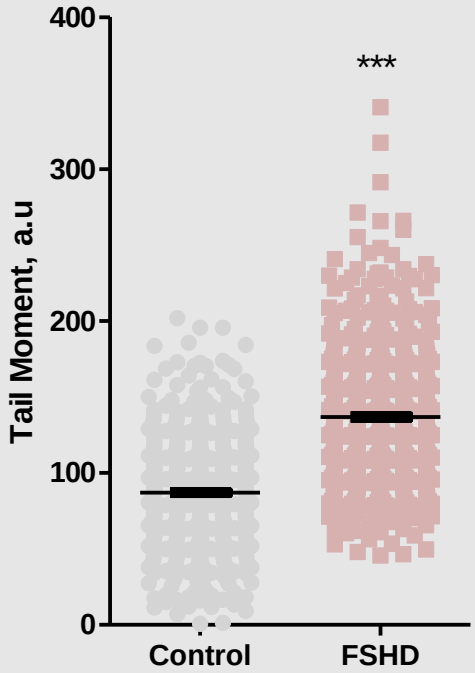
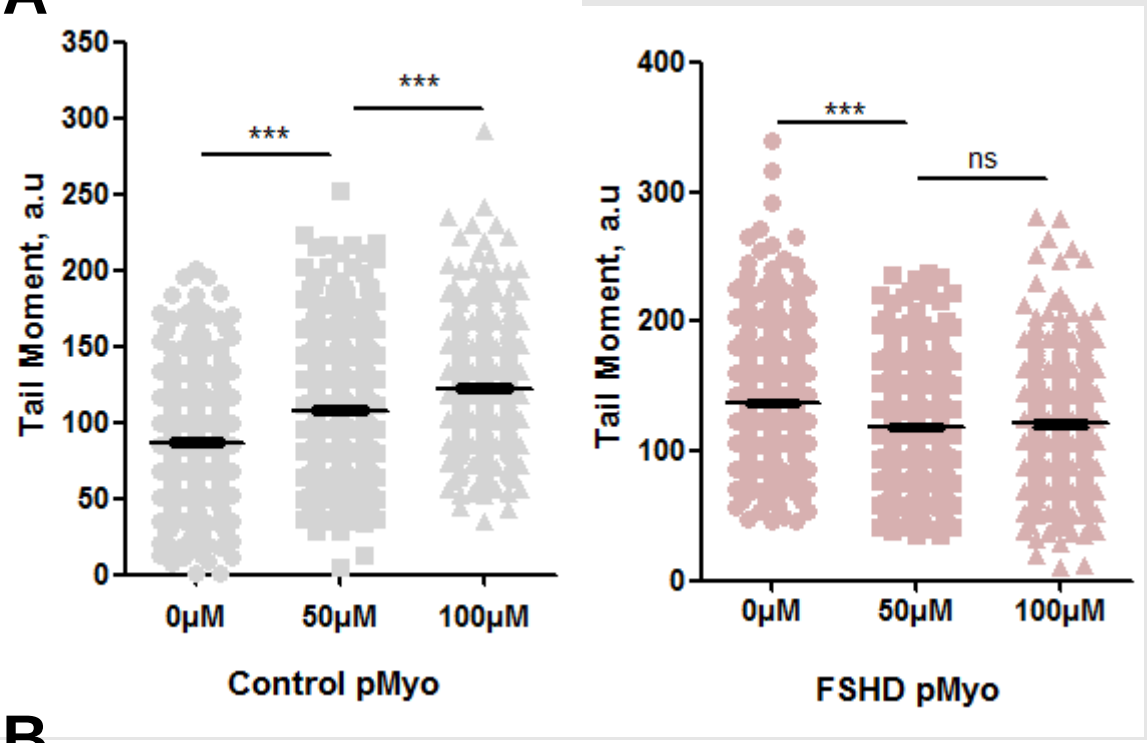


Figure 2

A



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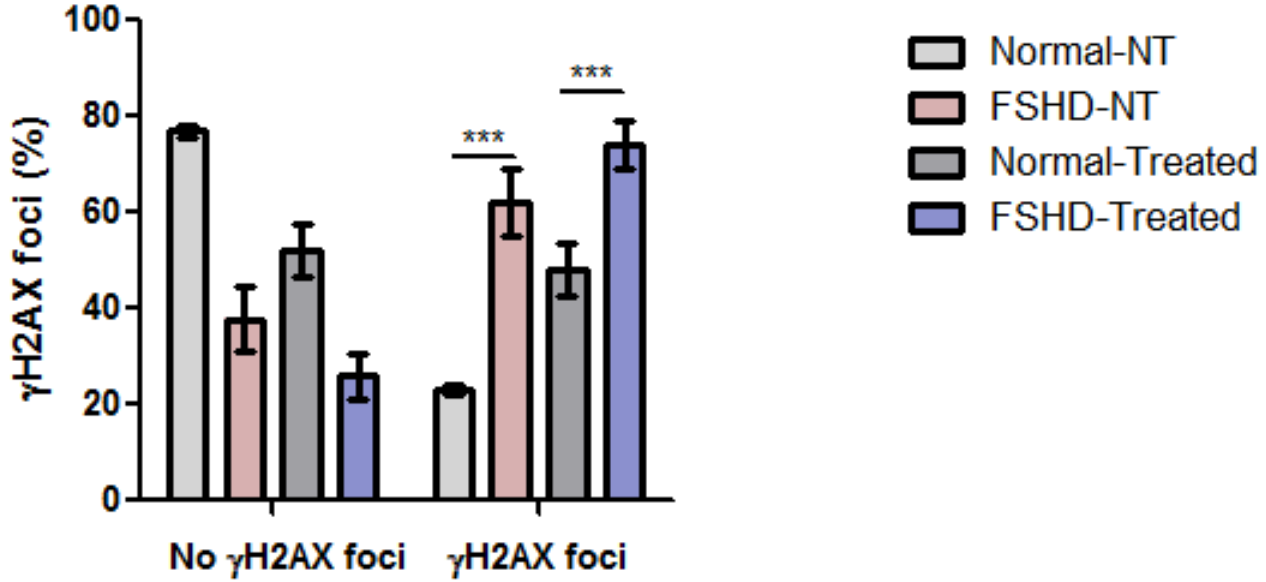


Figure 3

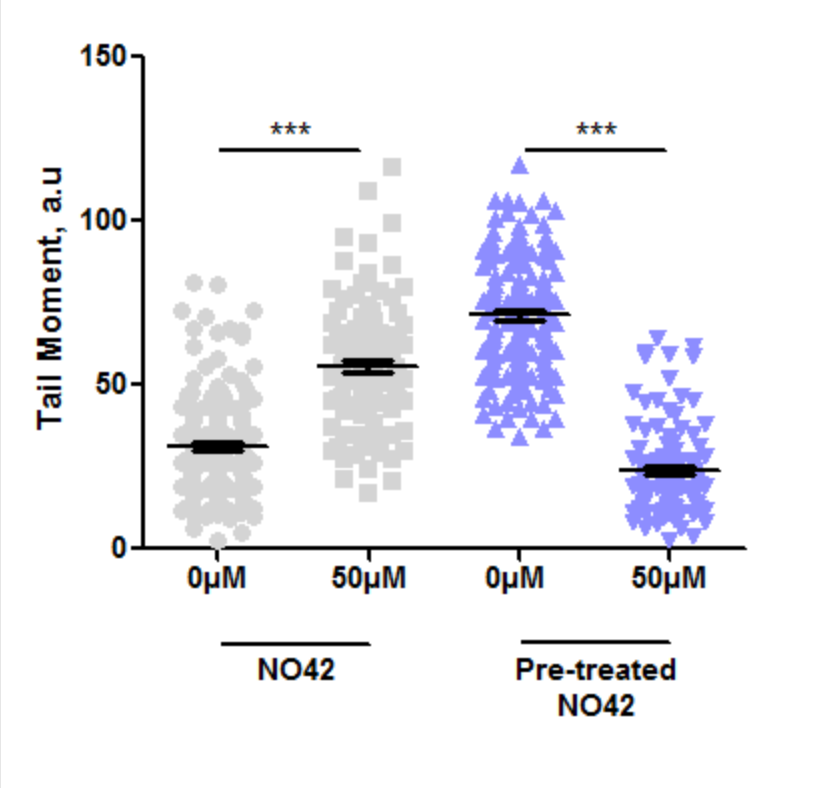
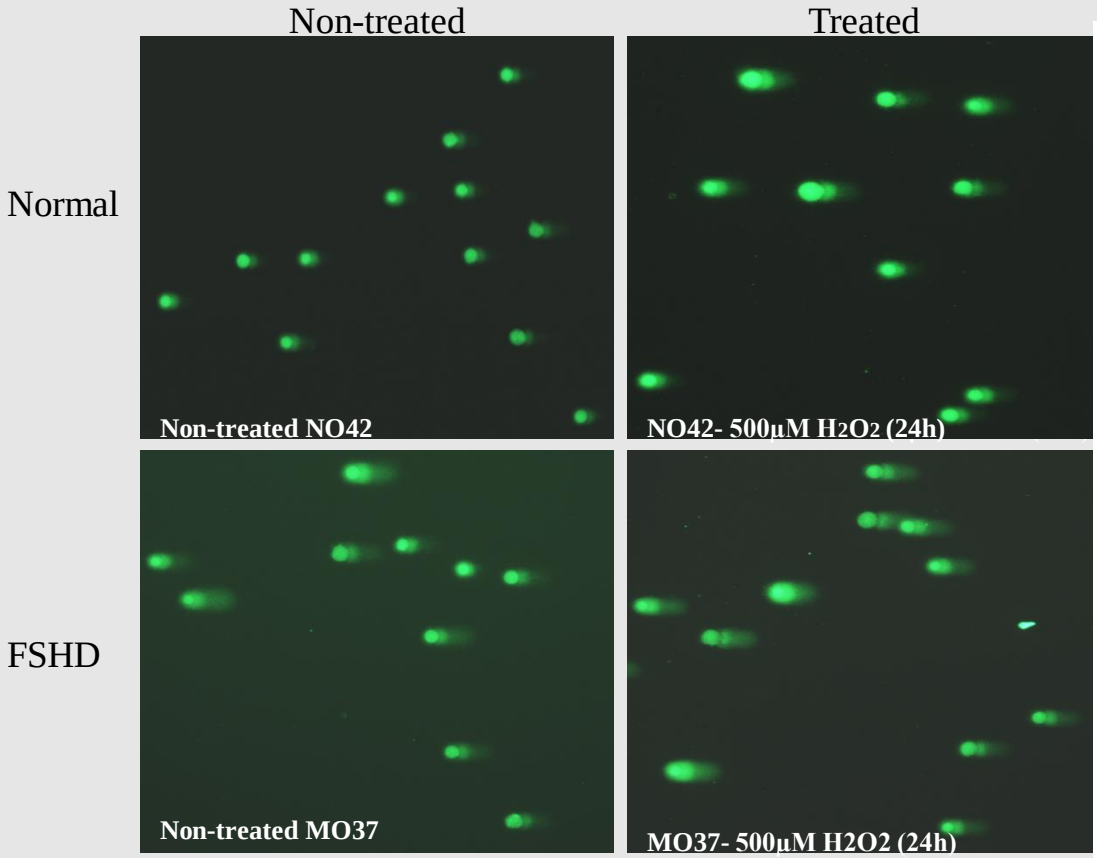


Figure 4


Non-treated
Treated
Normal
FSHD
Non-treated NO42
NO42- 500µM H2O2 (24h)
Non-treated MO37
MO37- 500µM H2O2 (24h)

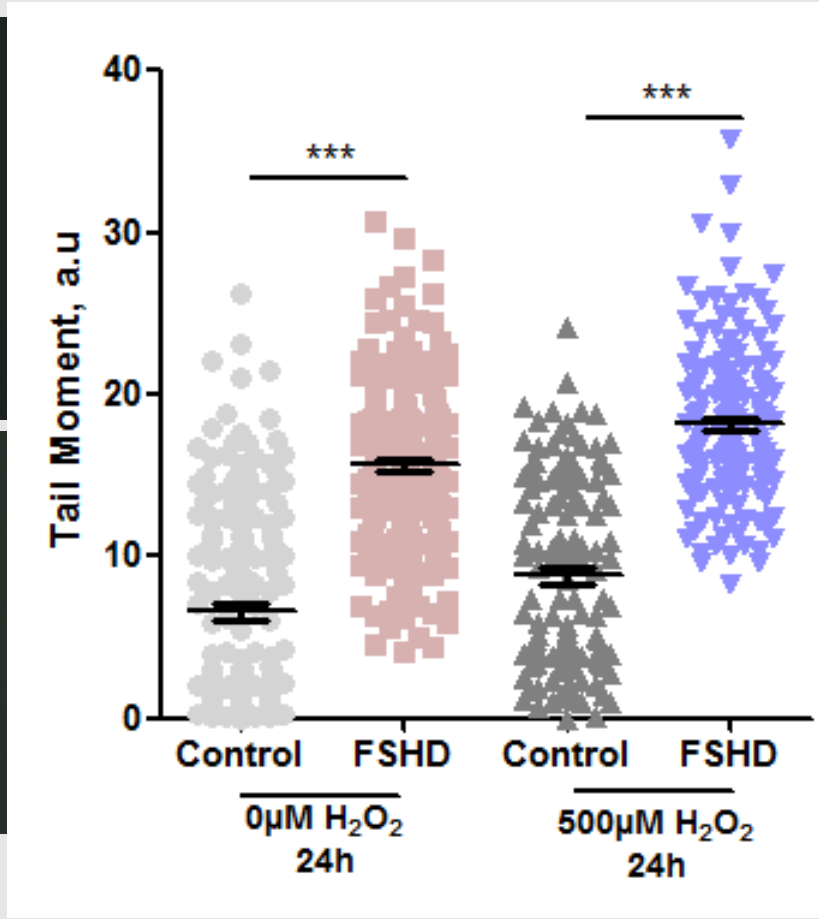
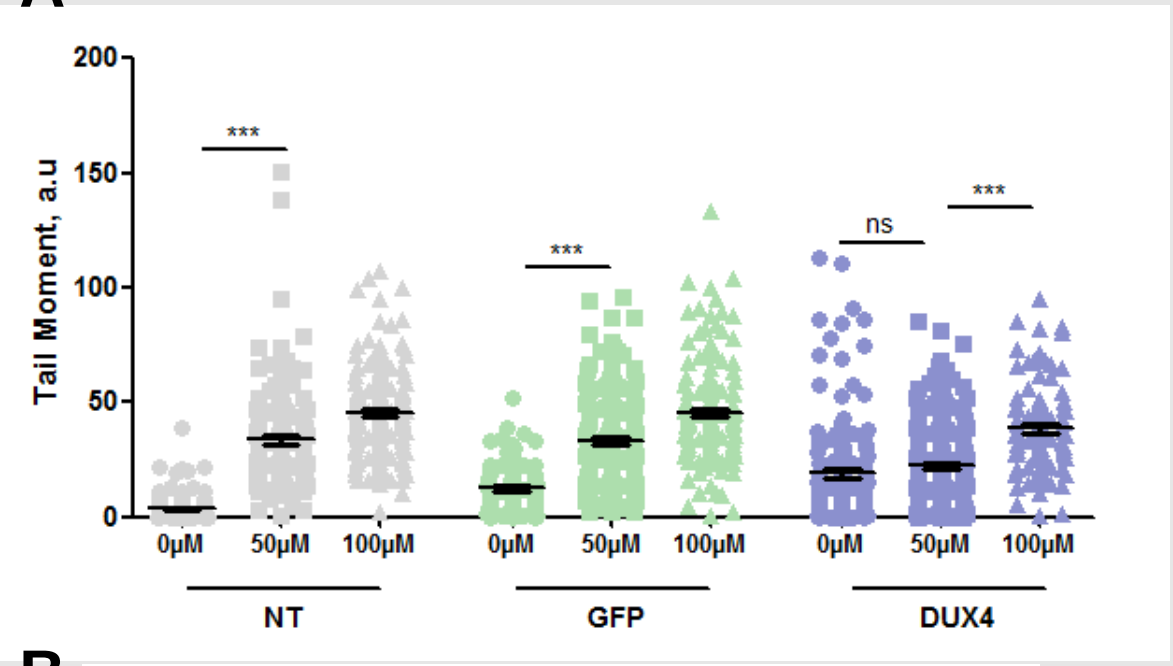
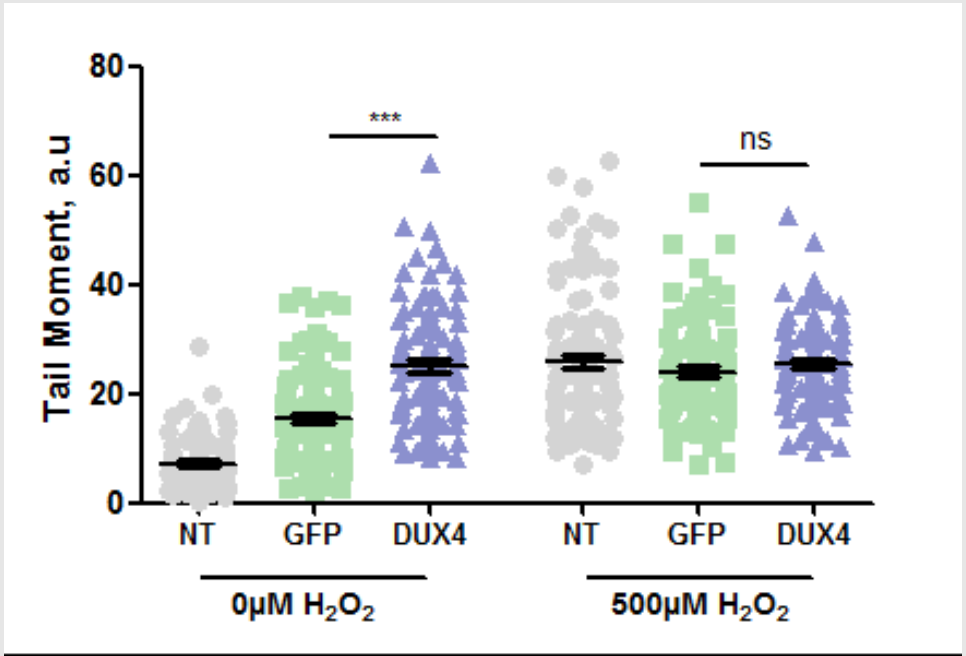

Tail Moment, a.u
40
30
20
10
0
***
***
Control
FSHD
Control
FSHD
0µM H2O2
24h
500µM H2O2
24h

Figure 5

A

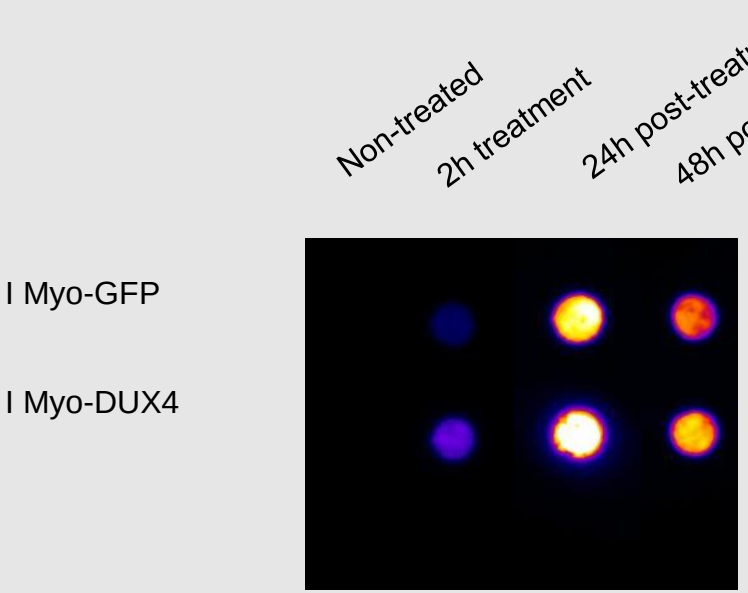


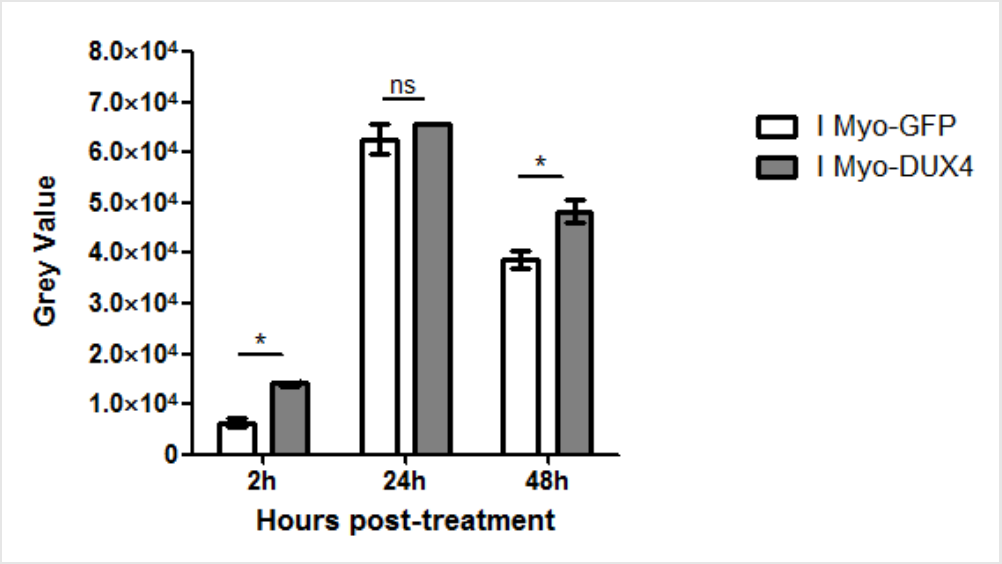
B



I-Myo DUX4 repaira less efficiently cisplatin adducts

Cisplatin treatment: 25µM , 2 hours
Assessment of DNA repaira efficiency 24h and 48h post-treatment





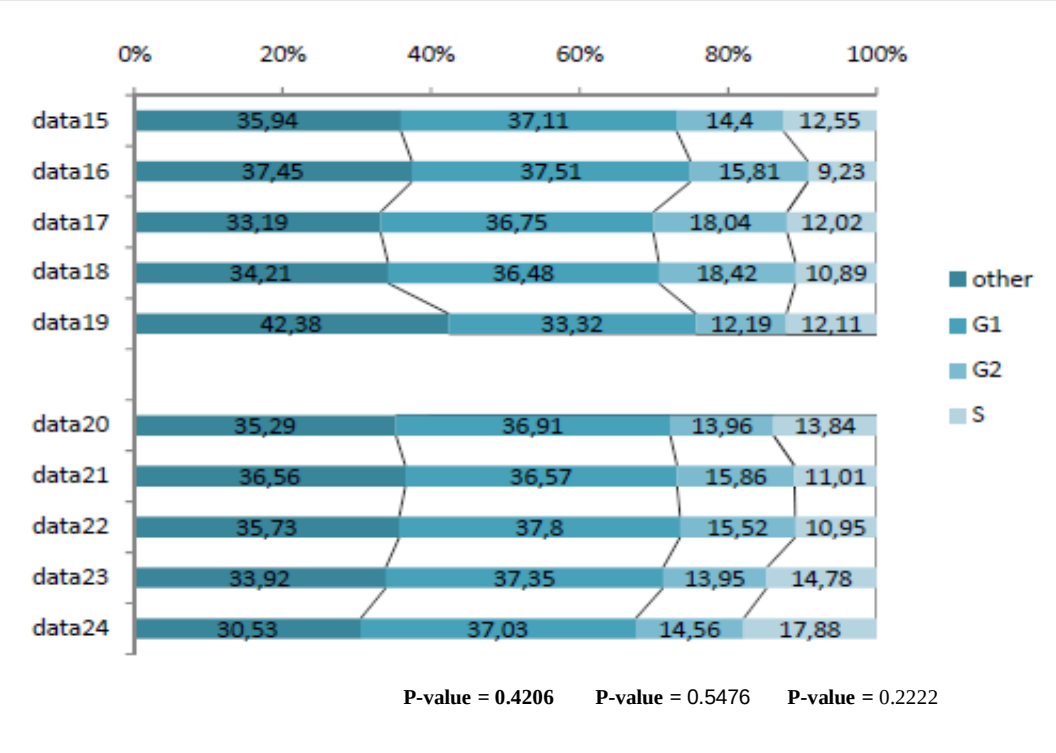
Cisplatin residues are more important in DUX4-expressing cells as compared to cells transfected with GFP

FSHD myoblasts demonstrate normal cell cycle distribution

the cell cycle distribution of FSHD cells was tested using BrdU labeling

Normal

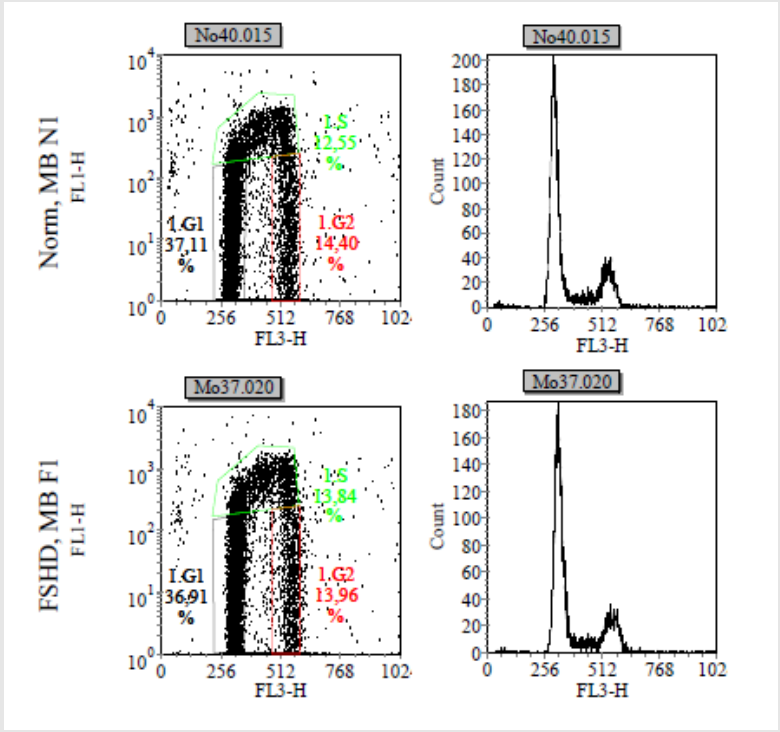
FSHD



No statistically significant differences in cell cycle stage distribution of FSHD cells as compared to normal myoblasts (Although several FSHD myoblast lines had higher percentage of S-phase cells)

FSHD myoblasts demonstrate normal cell cycle distribution

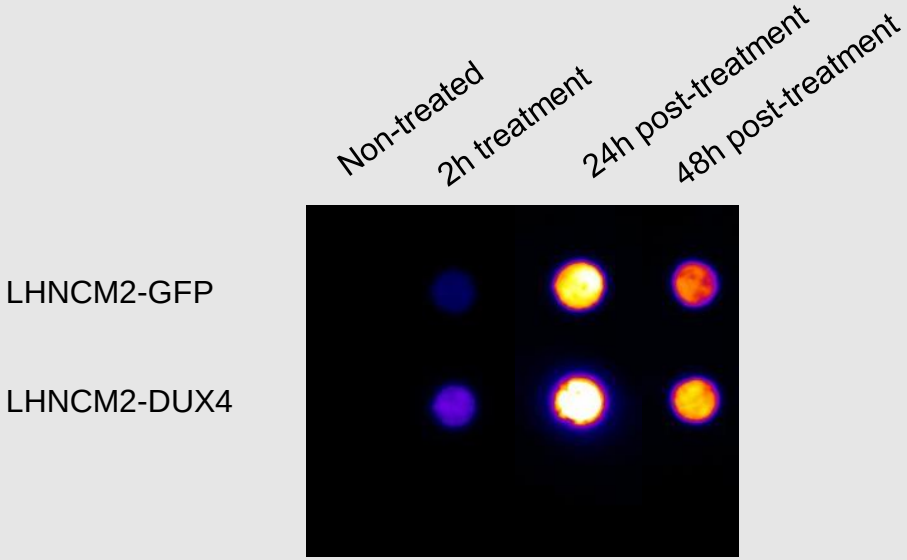
the cell cycle distribution of FSHD cells was tested using BrdU labeling



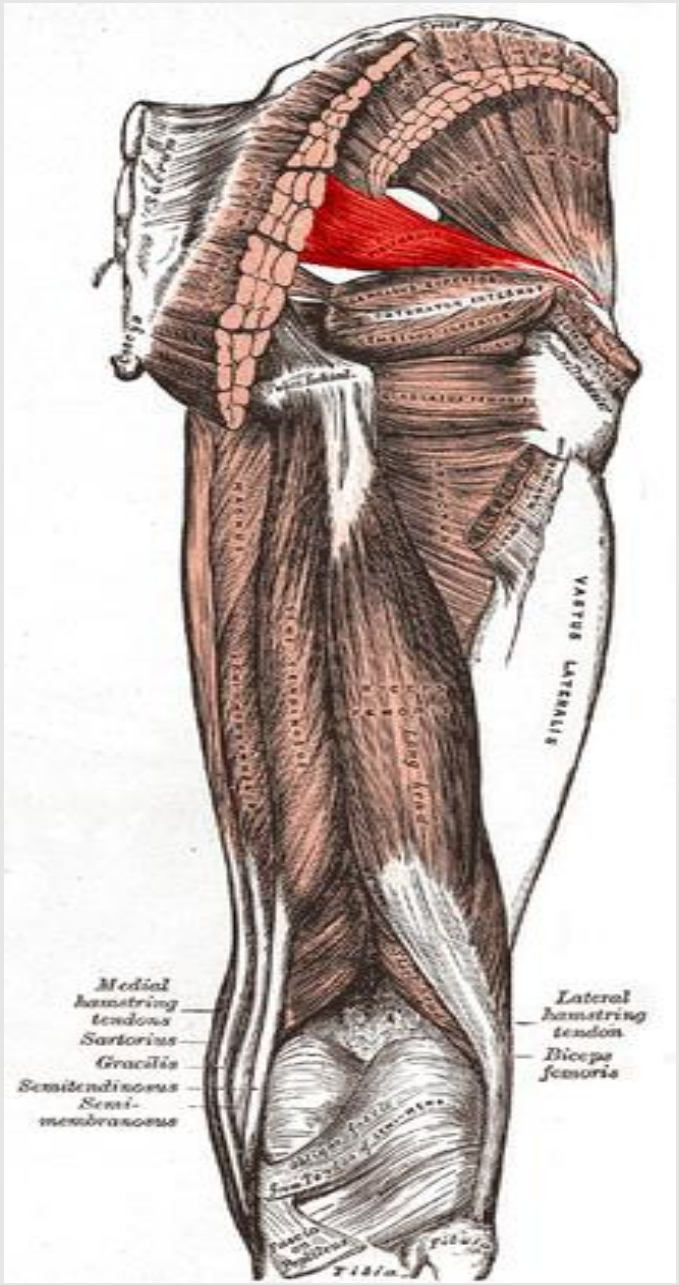
No statistically significant differences in cell cycle stage distribution of FSHD cells as compared to normal myoblasts (Although several FSHD myoblast lines had higher percentage of S-phase cells)

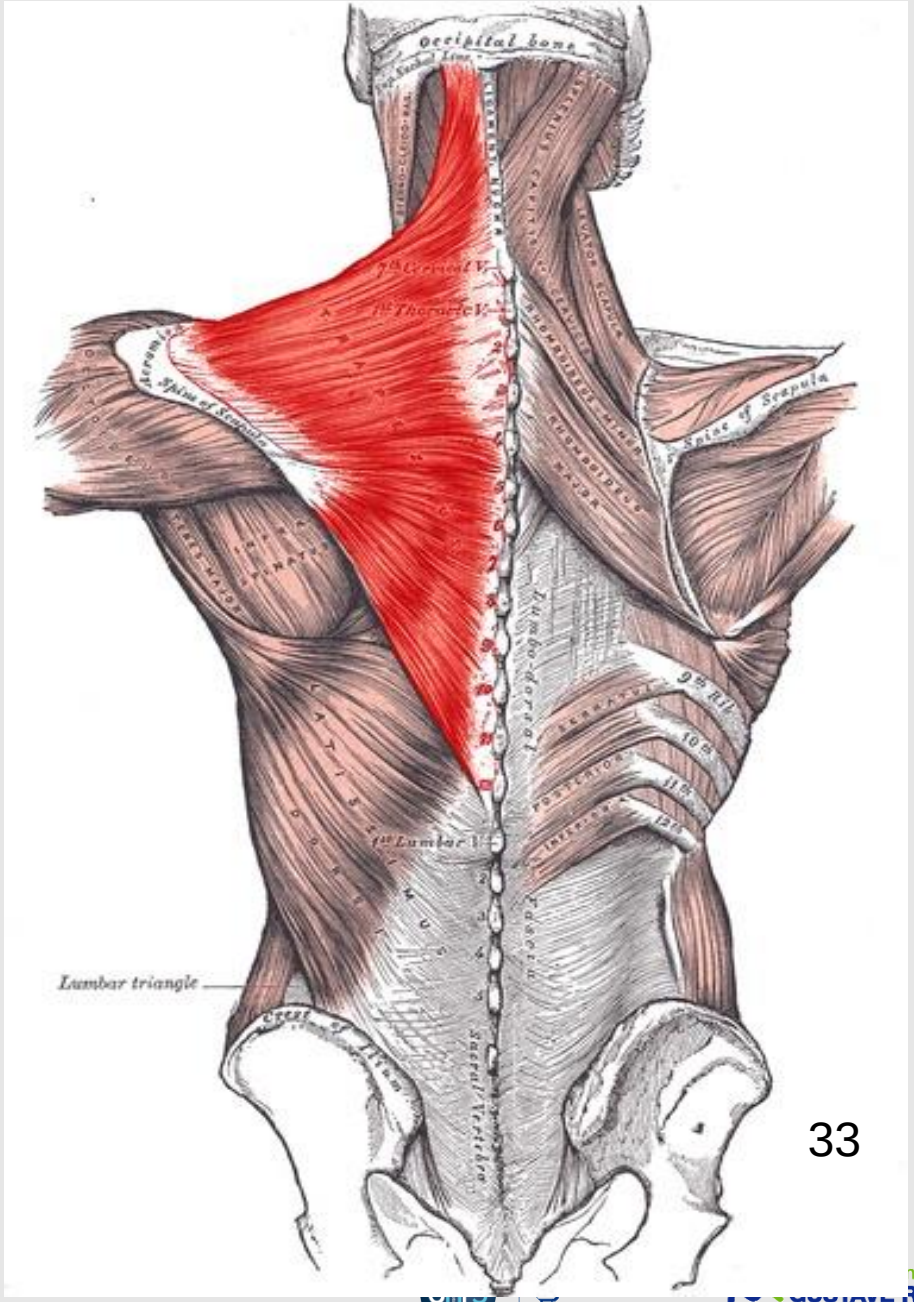
I-Myo DUX4 repaira less efficiently cisplatin adducts

Cisplatin treatment: 25µM , 2 hours
Assessment of DNA repaira efficiency 24h and 48h post-treatment

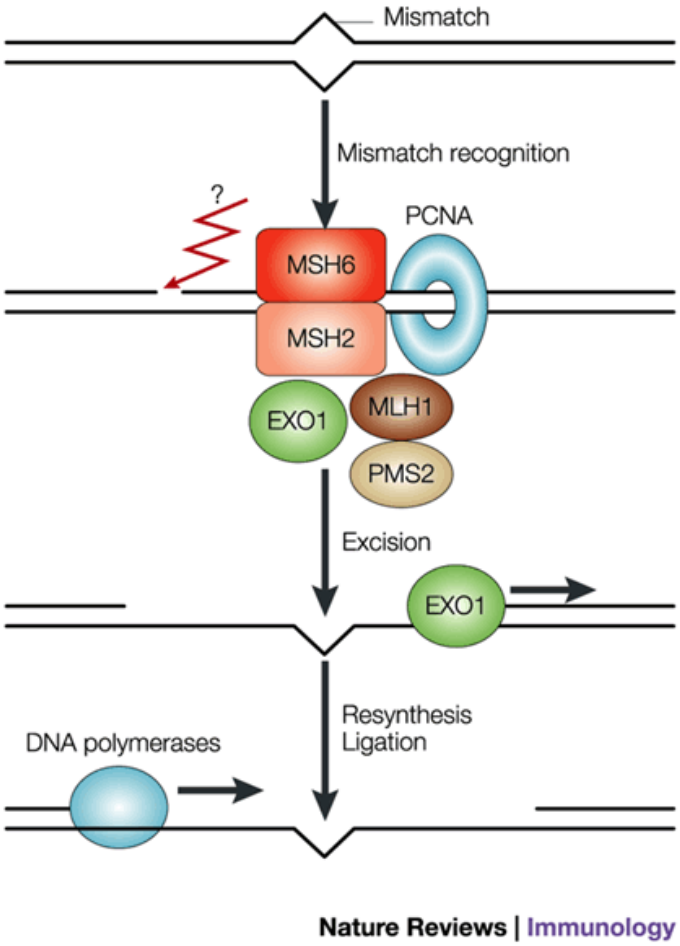


Cisplatin residues are more important in DUX4-expressing cells as compared to cells transfected with GFP


Medial hamstring tendons
Sartorius
Gracilis
Semitendinosus
Semi-membranosus
Lateral hamstring tendon
Biceps femoris
VASTUS LATERALIS

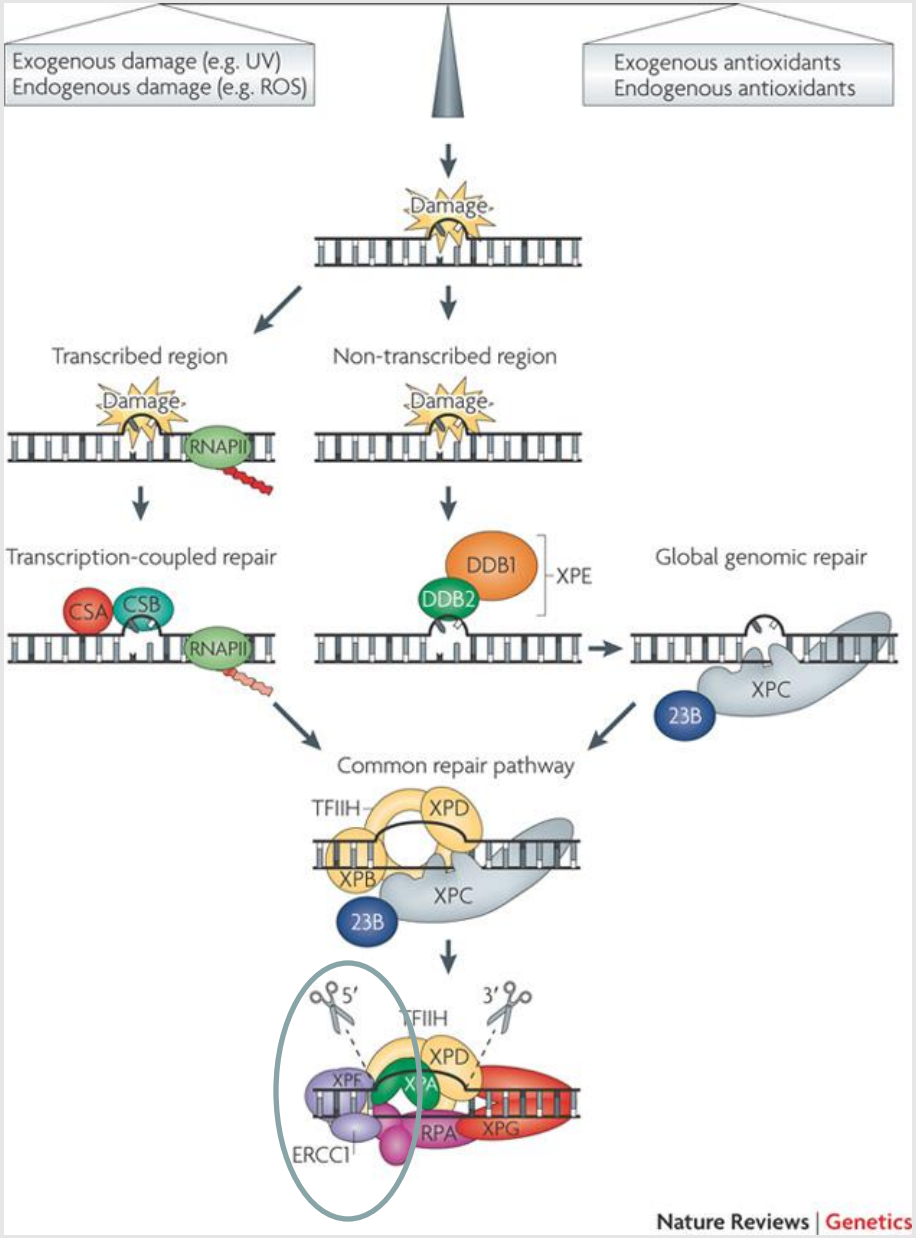

Occipital bone
Spine of Scapula
Lumbo-dorsal fascia
Lumbar triangle

Mismatch repair(MMR) – MSH6

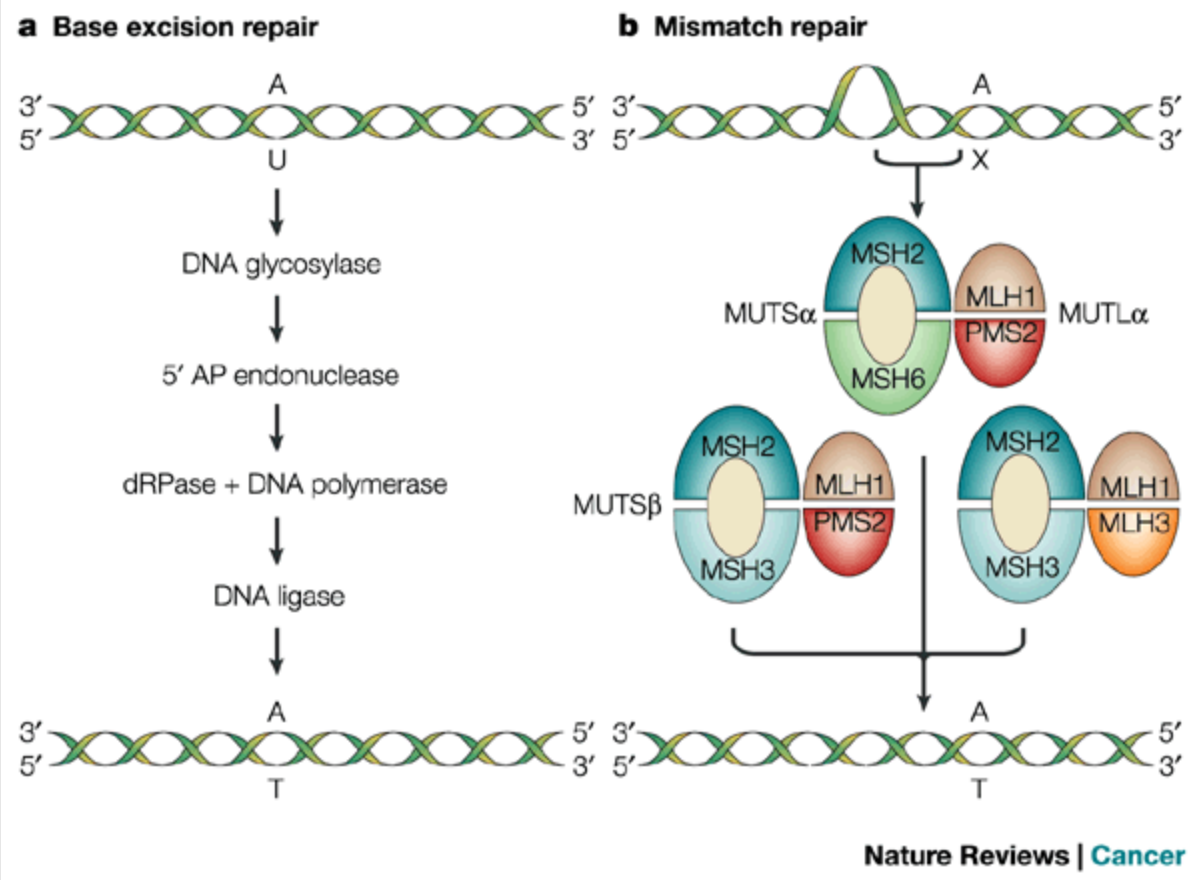


- MSH2–MSH6 heterodimers bind to single base-pair mismatches
- MMR might occur during DNA replication.
- Single-stranded DNA breaks occur during MMR
- The lesion is digested by exonucleases, such as EXO1, and then filled-in by translesional and/or replicative DNA polymerases.

Nucleotide excision repair (NER) – ERCC1

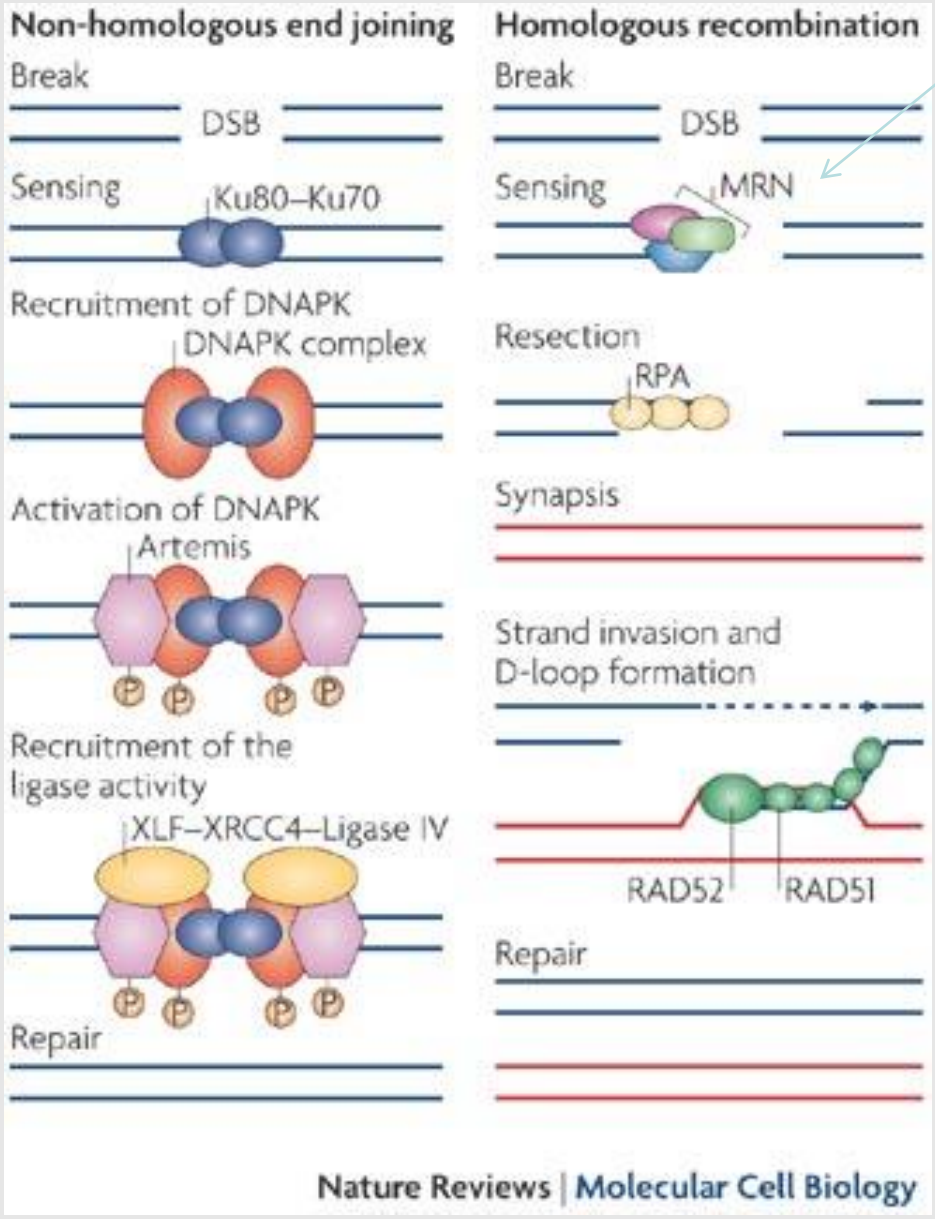


Base excision repair (BER)



DSB repair – HR/NHEJ

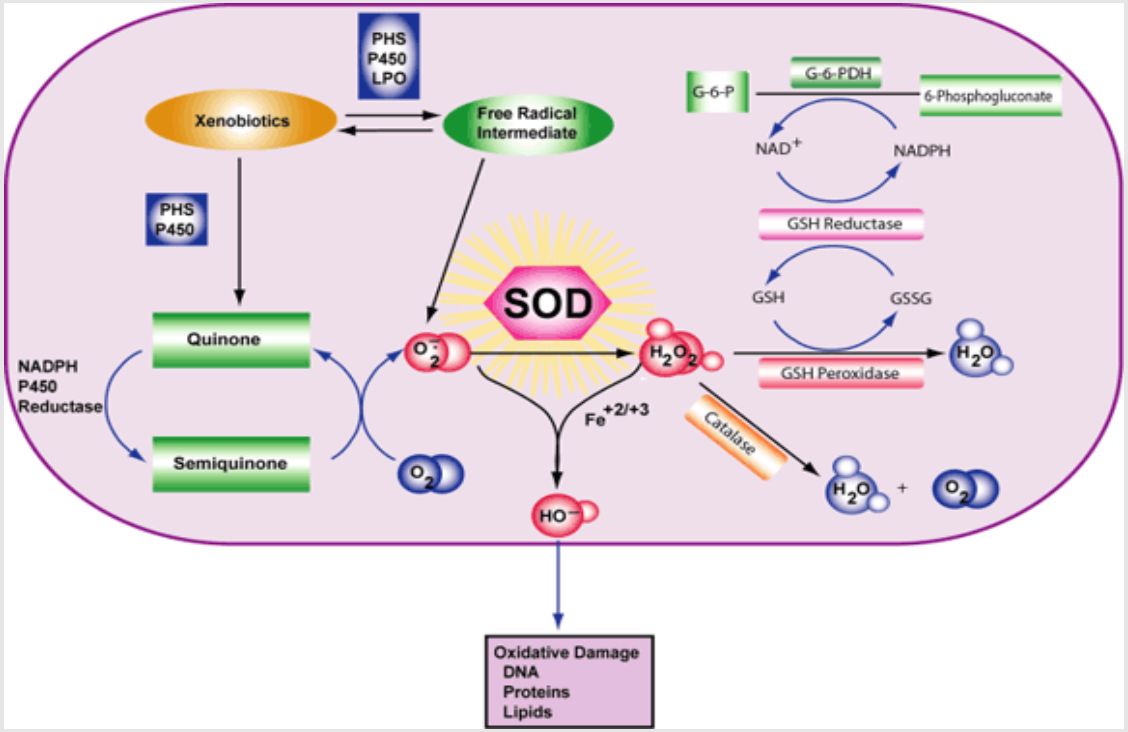
DSB is sensed by the Ku80–Ku70 heterodimer, which recruits the DNA-dependent protein kinase catalytic subunit DNAPKcs, resulting in assembly of the DNAPK complex and activation of its kinase activity → recruitment of XRCC4, DNA ligase IV, XLF and Artemis, which carry out the final rejoining reaction.

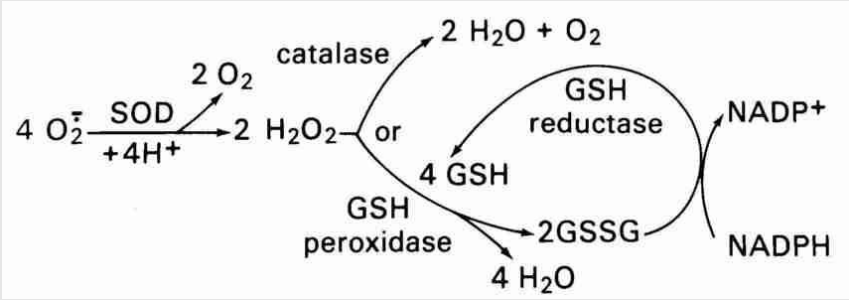


Rad50

DSB is recognized by the MRN (MRE11–RAD50–NBS1) complex, which is recruited to the DSB to generate single-stranded DNA by resection.The single-stranded ends are bound by replication protein A (RPA), RAD51 and RAD52 and can invade the homologous template, creating a D-loop and a Holliday junction, to prime DNA synthesis and to copy and ultimately restore genetic information that was disrupted by the DSB.

Cellular defense against the toxic effects of oxygen radicals





Fenton reaction:

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + \mathbf{OH^\circ}$$

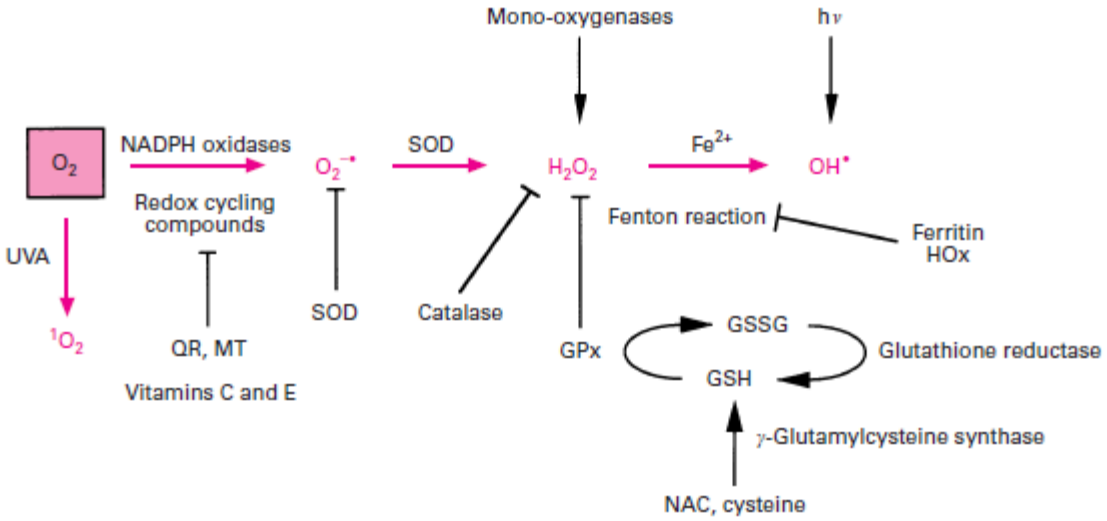


Figure 1 ROS generation and detoxification

Various chemical reactions, with or without enzymic catalysis, generate ROS. The dioxygen molecule undergoes successive reductions which yield the superoxide radical anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^\bullet$). Antioxidant systems act as ROS scavengers to maintain the intracellular redox status. Quinone reductase (QR) detoxifies quinone compounds, metallothionein (MT) traps (heavy) metal cations, and vitamins C and E trap free radicals. SOD and catalase respectively dismutate superoxide (into oxygen and hydrogen peroxide) and hydrogen peroxide (into oxygen and water). Glutathione peroxidase (GPx) acts like catalase on various peroxide compounds, including H_2O_2. The catalytic cycle of glutathione peroxidase involves the oxidation of GSH. GSSG can be reduced back to GSH by glutathione reductase. γ-Glutamylcysteine synthase is the limiting enzyme in the synthesis of GSH, and *N*-acetylcysteine (NAC) is a precursor of GSH. Haem oxygenase (HOx) catabolizes free haem structures, and the ferritin molecule traps Fe cations, which limits the deleterious Fenton reaction. hν, symbol for radiation energy.

Table 1 Genes that can undergo oxidative repression

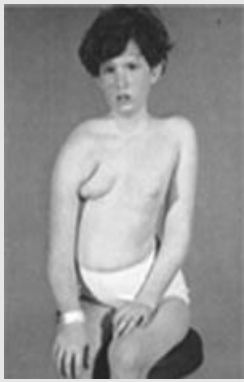
Endogenous gene	Redox modulator/ROS generator	Reference(s)
IL-2	H_2O_2 (extracellular)	45
	Xanthine oxidase activity	52
	Polyamine oxidase activity	53
TNFα (T cells)	H_2O_2 (extracellular)	54
CD3 (ζ chain)	H_2O_2 (extracellular)	56
CD16 (ζ chain)	H_2O_2 (macrophage-produced); diamide	57
Cyclins CLN1 and CLN2 (yeast)	O_2 (hyperoxia)	70,71
Glucokinase	O_2 (hyperoxia); H_2O_2 (extracellular)	72
PEPCK	H_2O_2 (extracellular)	75
Insulin	Glycation process	73
Tyrosine hydroxylase	O_2 (hyperoxia)	85
Tyrosine aminotransferase; tryptophan dioxygenase	Peroxidation products	79
pS2	H_2O_2 (extracellular)	84
CYP1A1	H_2O_2 (extracellular)	92
	Glutathione depletion; CYP1A1 activity	93
	TNFα; IL-1β	94
Several CYPs	Inflammatory cytokines	86–88
	Growth factors	89,90
Ferritin	H_2O_2 (extracellular)	100
	NO	105
EPO	H_2O_2 (extracellular)	108,109
	O_2 (normoxia and hyperoxia)	110
α-Actin; troponin I; myosin (light chain); creatine kinase (M isoform)	H_2O_2 (extracellular); glucose oxidase activity	114
Myosin creatinine phosphokinase (muscle)	NO; TNFα	113
Cytochrome *c* oxidase	H_2O_2 (extracellular); catalase knock-out	64

Table 2 Transcription factors that can undergo oxidative repression

Abbreviations: SV40, simian virus 40; NLS, nuclear localization signal; PEBP2/CBF, polyoma virus enhancer-binding protein 2/core binding factor.

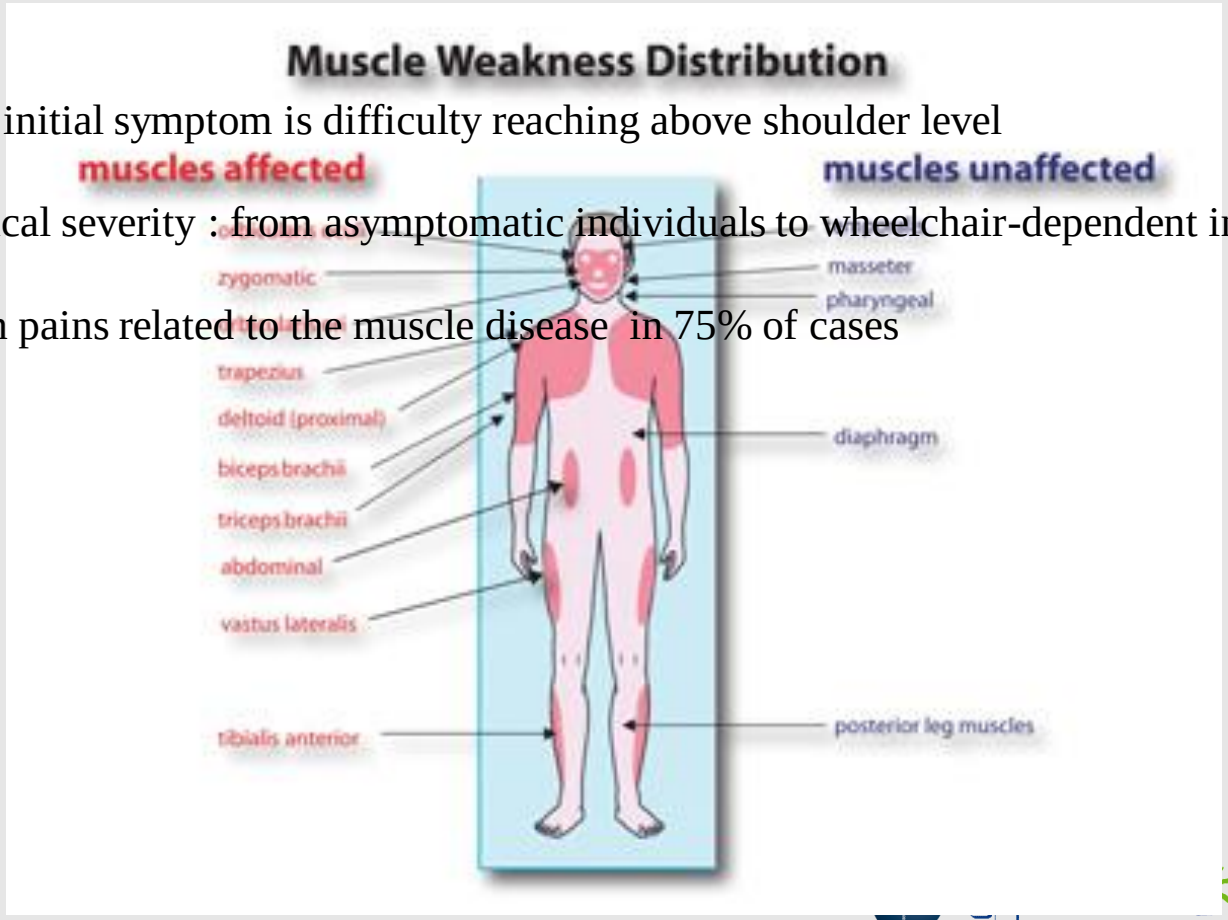
Transcription factor	ROS target	Related gene redox regulation	Reference(s)
Sp1	DBD (Cys_2His_2 zinc fingers)	SV40 (viral promoter); β-enolase; dihydrofolate reductase	129–132
NFI	Several cysteines within the DBD	CYP1A1	126
	A cysteine within the TAD	CYP1A1	93
GR	Cysteines within the DBD	Tyrosine aminotransferase	76–78
	Cys-481 within the NLS	Tryptophan dioxygenase	49,82
ER	Cysteines within the DBD	pS2	83,84
USF	Cys-229 and Cys-248 (DBD)	–	135
MyoD	Cys-135	–	137
HIF-1α	Cys-774 (TAD)	EPO	139,141,143,144
PEBP2/CBF	Cys-124 (DBD)	–	159
AP-1 (Jun)	Cys-252 (DBD)	–	160
AP-1 (Fos)	Cys-154 (DBD)	–	160
NF-κB (p50)	Cys-61 (DBD)	–	167,168
p53	Several cysteines within the DBD	–	148,149,151,152

FSHD – Clinical manifestations



•Progressive muscle weakness and asymmetric muscle impairment

• FSHD is characterized by onset of weakness in a characteristic distribution:
facial weakness→scapular fixator →humeral → truncal →lower-extremity weakness



•The most common initial symptom is difficulty reaching above shoulder level

• Wide ranging clinical severity : from asymptomatic individuals to wheelchair-dependent individuals

• Muscle and tendon pains related to the muscle disease in 75% of cases

FSHD – Current treatments

No disease-specific therapeutic strategies :

→Non steroidal anti-inflammatory drugs to relief the pain

→ Exercise: strength training and aerobic exercise (depending on individual disease severity)

→Surgical interventions (eg. Scapular fixation)

→Pharmacological strategies have been tested
- Corticosteroids
- Creatine monohydrate
- Myostatin inhibition
- Folic acid
- Methionine supplementation) …

No benefit on muscle function or strength

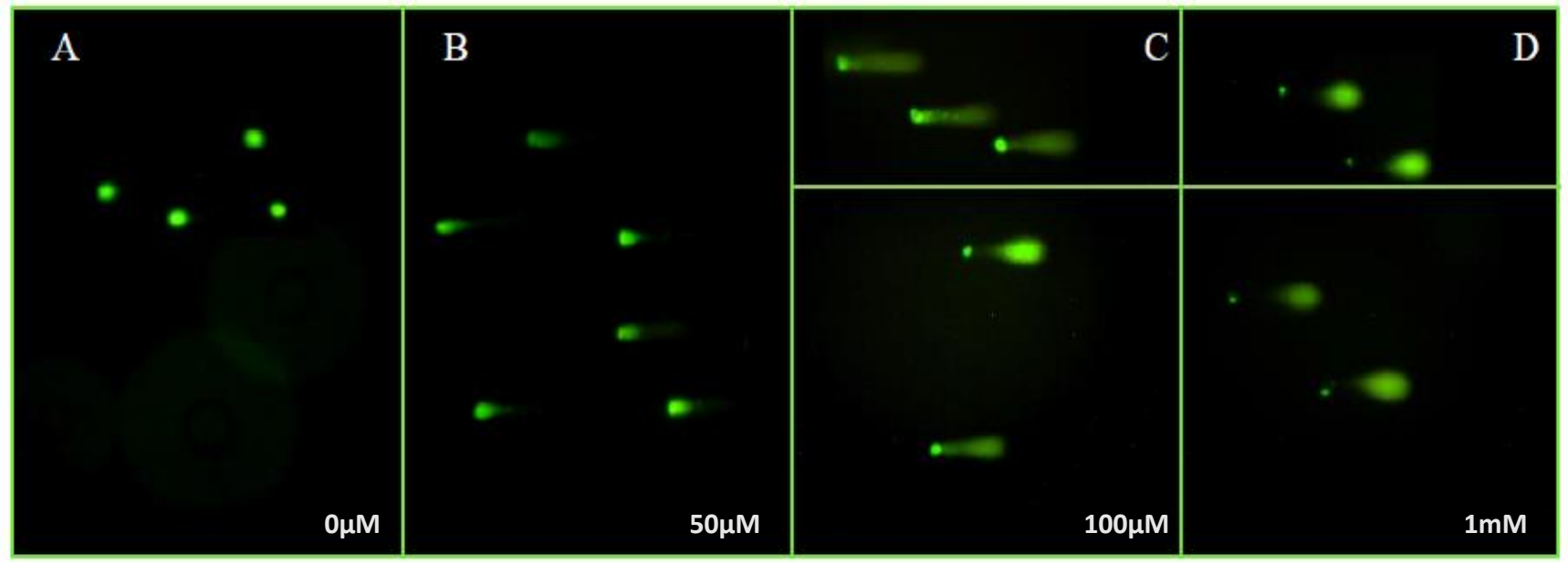
→Autologous muscle stem cell therapy ?

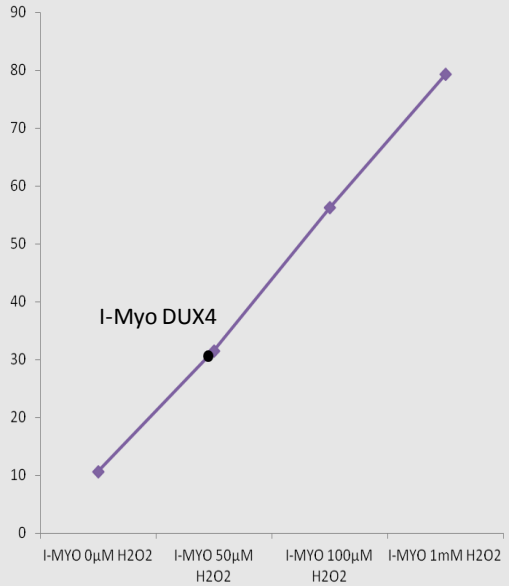
DNA damage and DNA repaira efficacy in FSHD

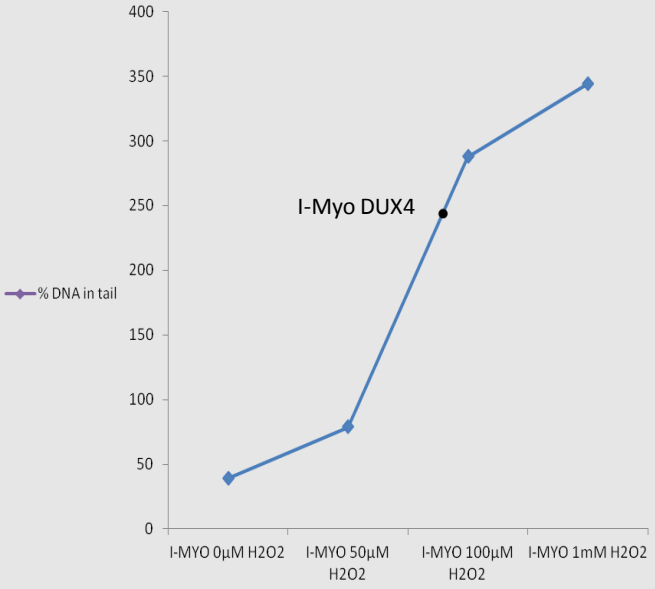
Primary myoblasts derived from FSHD patients used in this study

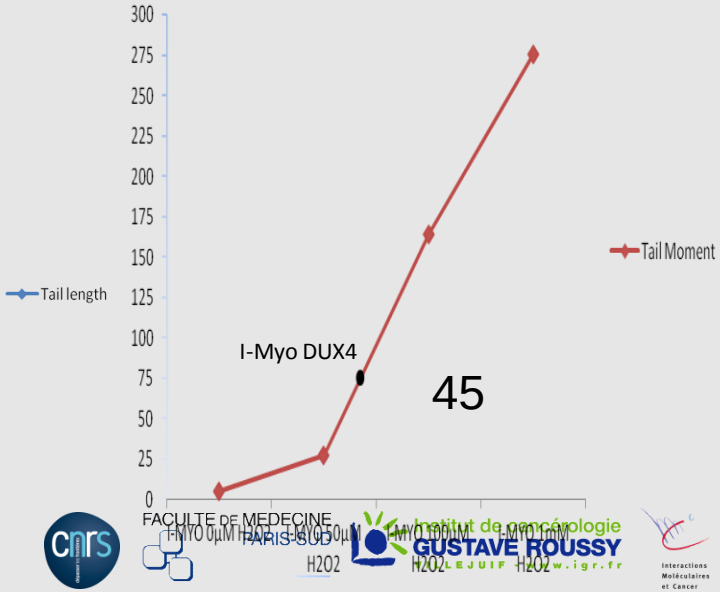
Name	Sex	Age (years)	D4Z4 copy number	Muscle	Brooke scale ARMS	Vignos scale LEGS
FSHD1	M	30	5	Trapezius	4	5
FSHD2	F	54	5	Piriformis	3	4
FSHD3	F	32	7	Vastus lateralis	1	1
FSHD4	M	41	7	Infraspinatus	4	4
FSHD5	M	53	6	Vastus lateralis	2	3
FSHD6	F	23	8	Vastus lateralis	1	1
FSHD7	M	53	9	Femoral biceps	2	2
FSHD8	M	39	6	Vastus lateralis	2	1
FSHD9	M	36	7	Vastus lateralis	2	1
FSHD10	F	20	4	Vastus lateralis	1	1
FSHD11	M	44	7	Vastus lateralis	3	3
FSHD12	F	38	7	Vastus lateralis	1	1
FSHD13	F	42	8	Vastus lateralis	4	3
FSHD14	M	25	4	Vastus lateralis	1	1

DUX4 overexpression induces an increase in DNA damage in I-Myo





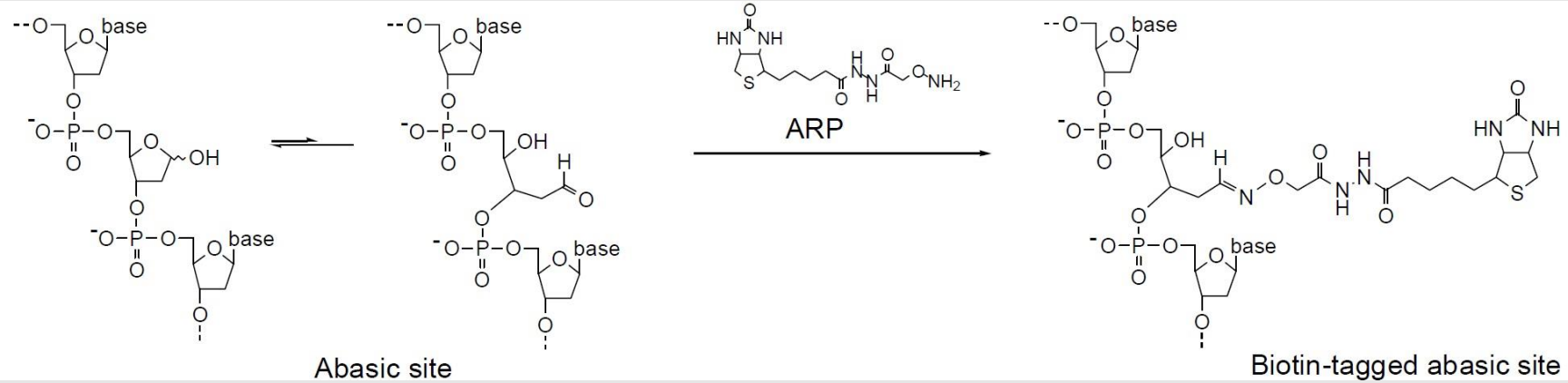


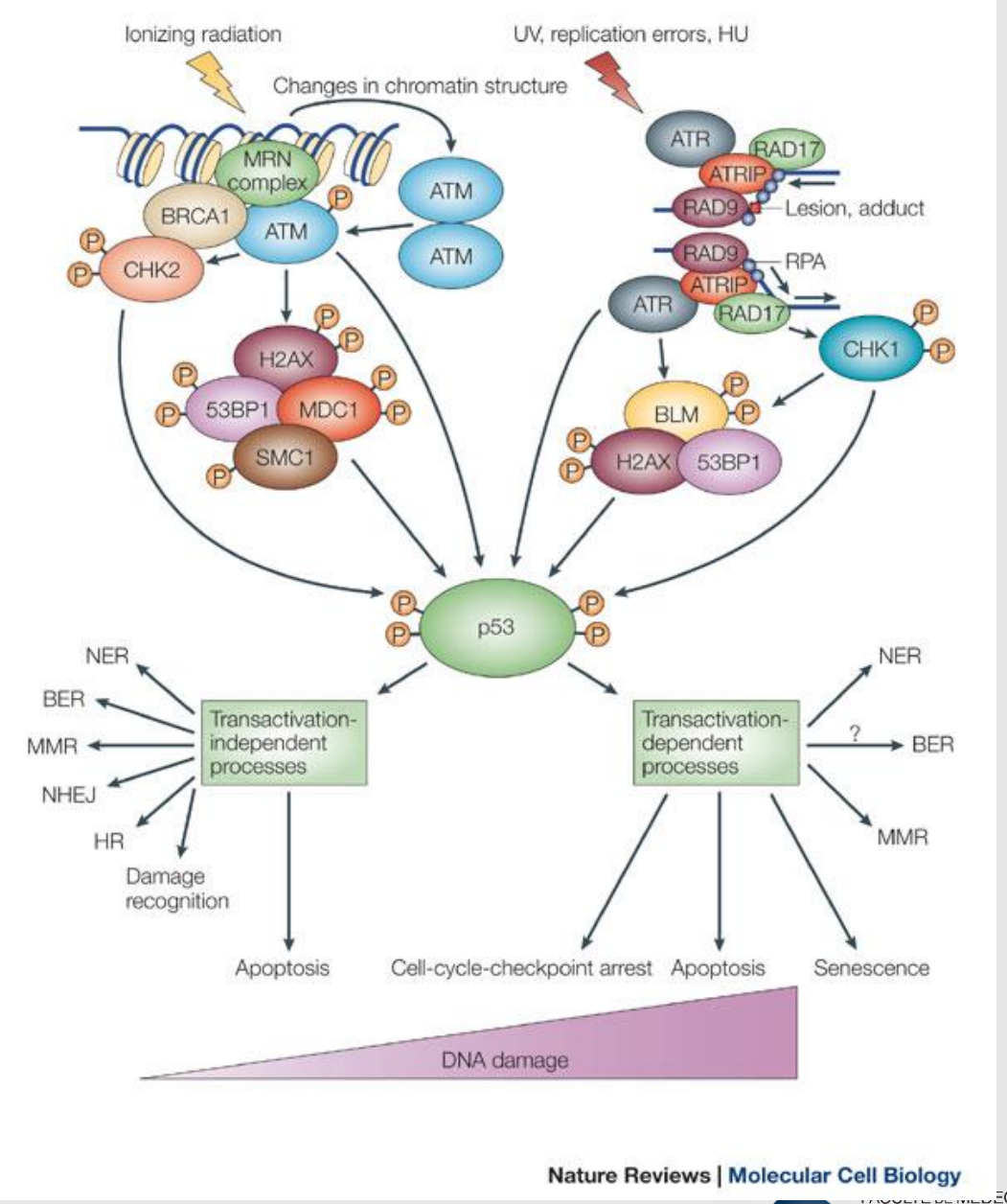


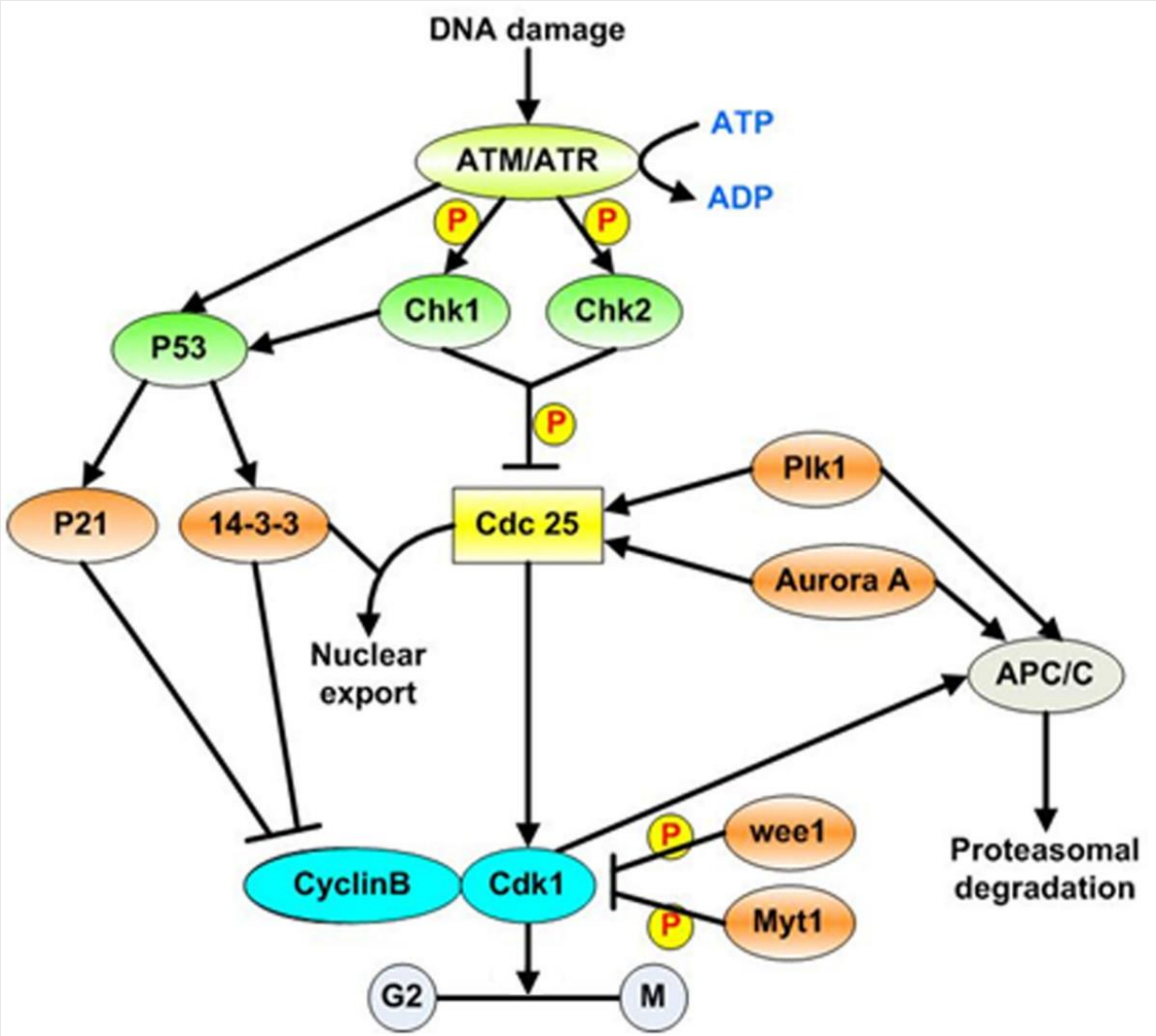
AP sites

Oxidative attacks by hydroxy radicals on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic sites (AP sites). In fact, AP sites are one of the major types of damage generated by ROS. Aldehyde Reactive Probe (ARP; N'-aminooxymethylcarbonylhydrazin-D-biotin) reacts specifically with an aldehyde group present on the open ring form of the AP sites.

The loss of DNA bases and attendant formation of AP sites in DNA occurs spontaneously as a result of hydrolytic cleavage of *N*-glycosylic bonds. AP sites are also generated through glycosylase-catalyzed removal of damaged bases during the early stage of base excision repair (BER)




Ionizing radiation
UV, replication errors, HU
Changes in chromatin structure
MRN complex
BRCA1
ATM
ATM
ATM
CHK2
ATR
RAD17
ATRIP
RAD9
Lesion, adduct
RAD9
RPA
ATRIP
ATR
RAD17
CHK1
H2AX
53BP1
MDC1
SMC1
BLM
H2AX
53BP1
p53
NER
BER
MMR
NHEJ
HR
Damage recognition
Transactivation-independent processes
Transactivation-dependent processes
NER
?
BER
MMR
Apoptosis
Cell-cycle-checkpoint arrest
Apoptosis
Senescence
DNA damage
Nature Reviews | Molecular Cell Biology


DNA damage
ATP
ATM/ATR
ADP
P
P
Chk1
Chk2
P53
P
P21
14-3-3
Cdc 25
Plk1
Aurora A
Nuclear
export
APC/C
P
wee1
CyclinB
Cdk1
P
Myt1
Proteasomal
degradation
G2
M

Pathways involved in mediating cisplatin-induced cellular effects

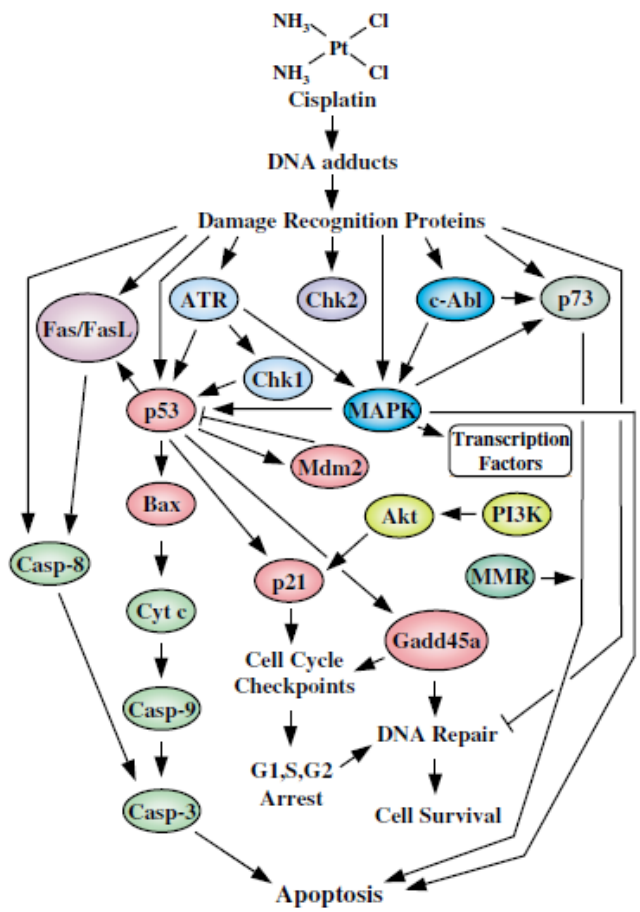


Figure 1 An overview of pathways involved in mediating cisplatin-induced cellular effects. Cell death or cell survival will depend on the relative intensity of the signals generated and the crosstalk between the pathways involved. Some of the signaling discussed in the text has been omitted for clarity

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- **Carla Dib**, doctorante

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